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# Canadian Journal of Research

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VOL. 15, SEC. C.

JANUARY, 1937

NUMBER 1

# THE INFLUENCE OF BIOS ON NODULE BACTERIA AND LEGUMES<sup>1</sup>

A. THE INFLUENCE OF BIOS ON LEGUME SEEDLINGS

By D. G. LAIRD<sup>2</sup> AND P. M. WEST<sup>3</sup>

#### Abstract

The hypocotyls of red clover seedlings, when sprouted on a seed bed enriched with crude Bios 2, grew upwards in a vertical position, while the cotyledons rested on the surface, supporting the inverted plant and possibly absorbing nutrients for its growth. From the tip of the upturned primary root, secondary roots developed about ten days after seeding. These new roots grew downwards, and after approximately one week, they penetrated the substratum in a normal fashion.

The concentration of crude Bios 2 necessary to cause maximum hypocotyl bending, was observed to be approximately four times that required to produce optimum stimulation of the nodule bacteria.

When plants were allowed to start in an unenriched growing medium superimposed on a layer of agar enriched with crude Bios 2, no upward bending of the roots occurred as they reached the Bios layer.

Bios 2(b) alone appears to be the factor responsible for the hypocotyl bending phenomenon. Bios 1, Bios 2(a), pantothenic acid, various amino acids, and miscellaneous compounds were tested and did not appear active in this respect.

Though causing a different form of root aversion, hetero-auxin, like Bios 2(b), actually prevents the hypocotyls from entering an enriched medium. While the two substances bring about a certain similarity of physiological effect, they cannot be considered identical, and chemically, they are absolutely distinct.

A drop of Bios 2(b) placed on the sensitive parenchymous lining of a bean pod caused rapid cell multiplication, resulting in the production of a wart-like protuberance. Similar, though somewhat less distinct results followed the pricking of such tissue with a pin. These results would appear to strengthen the hypothesis advanced by Went in which Bios was assigned the properties of a "wound hormone".

A study devoted to the influence of the Bios complex on nodule bacteria, alone and in association with red clover (unpublished data), suggested to the writers that Bios might exert a specific influence on the character of the host. While the Bios of Wildiers is commonly known to occur in the tissues of higher plants (4, 5, 11), its effects on the growth processes of such plants are as yet unknown. Kogl (6), has suggested that Bios may be physiologically related to auxins (a) and (b), and hetero-auxin, which appear to function both as organizators and as phyto-hormones of cell expansion. Went (12), on the other hand, has presented the hypothesis that Bios might be considered

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a wound hormone. A consideration of these facts and suggestions, and a desire for increased knowledge regarding the important interrelationships between bacteria and legumes have been the inducements for a study, as reported below, of the influence of Bios on the growth of red clover seedlings.

The Bios fractions used in preliminary work by Eagles *et al.* (3), were prepared after the manner of Miller and his associates (9). As the work progressed, however, the original procedure for the isolation of Bios concentrates was altered in order to obtain fractions with fewer impurities. These efforts resulted in the development, by Eagles and Wood (unpublished data), of an improved procedure. The Bios 2(a) and 2(b) concentrates prepared according to the new method were found to exercise the same biological effects as the respective fractions prepared after the manner of Miller when tested on bacteria, but possessed a distinctly greater activating capacity. Bios obtained by the revised procedure of fractionation was used in the plant studies subsequently reported upon.

The following method was employed to determine the influence of Bios 2 preparations on the growth of red clover: Using washed sand cultures, the seeding was carried out under aseptic conditions; the required nutrients were supplied in the form of modified Crone's solution (2), containing, except in the case of the controls, 2% of an active solution of Bios 2, *i.e.*, a solution containing both Bios 2(a) and Bios 2(b). The rate of germination was normal, but the seedling roots growing on a Bios medium absolutely refused to penetrate the sand, with the result that they were unable to support the plant. After the surface of the sand had dried slightly, the plants wilted and died. This experiment was conducted in 4-inch porcelain pots, each treatment replicated four times, and about 50 seedlings grown in each pot. The test was repeated to guard against any possible error.

As sand did not appear to constitute a satisfactory growing medium, it was replaced by a modified Crone's nutrient solution to which was added 0.75% Bacto agar. The Bios enrichment used was the same as in the previous experiment. Red clover seeds were then placed on the surface of the medium which had been autoclaved in quart sealers covered by a Petri plate. The effect of the Bios became evident after three to four days. In every case the hypocotyls ascended vertically or nearly so, while the cotyledons remained in contact with the agar surface (see Fig. 1). This phenomenon is referred to throughout the remainder of the paper as "root bending", since it is the root tissue of the hypocotyl that undergoes subsequent modification and not the stem tissue. The upturned hypocotyls differed further from the normal in being slightly swollen and in possessing no root hairs. The upward growth continued for approximately ten days, and after that, one or more secondary roots were produced which grew downwards and penetrated the substratum in an apparently normal fashion. In most cases, the first true leaf and the subsequent trifoliate leaf had developed when the secondary roots entered the nutrient medium (see Fig. 2). Until this time, the absorption of water and any possible nutrients required must have taken place through the coty-

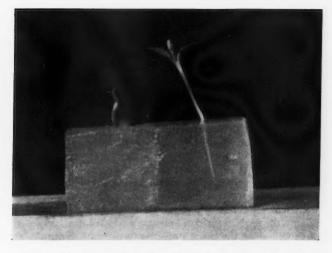


Fig. 1. Red clover seedlings 7 days old. Left: typical upward growth of hypocotyl on medium enriched with Bios 2. Right: normal growth on unenriched control medium.

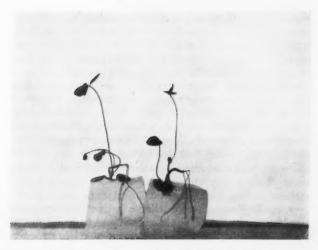


Fig. 2. Red clover seedlings 21 days old, on Bios 2 enriched medium. Note secondary roots growing down in a normal fashion through the medium which caused hypocotyls to bend upwards.

ledons, or the stem immediately beneath them, or through both. Thousands of seedlings have been used in this study. The experiment has been repeated numerous times and always with identically the same result. In fact, in work now in progress, the hypocotyl behavior as described is being used in determining the character of certain fractions as isolated from plant and animal materials.

It became evident, during the investigation, that red clover seedlings vary in their response to Bios enrichment, and this observation led to a detailed study of the effect of different Bios concentrations. The concentration of any preparation required for maximum "root bending" was observed to be roughly four times that required to produce an optimum stimulation of the nodule organism. Fractions of unknown activity were, therefore, first tested on bacteria to obtain an indication regarding the amount required to produce "root bending". By varying the concentration of Bios in the medium from 0.25 to 8% the maximum "root bending" effect was observed to occur with a 2% enrichment. Beyond 2% there was evidence of slight toxicity, while below 2% only the more "sensitive" plants responded, the roots of the others merely extending along the surface of the substratum. Since the activity of different Bios solutions may, and do, vary to some extent, the above figures refer only to the preparation used in these experiments. While a positive correlation was observed to exist between the Bios concentrations required for optimum bacterial growth and "root bending", the possibility is not to be overlooked that more than one factor might be concerned.

Likewise, it seemed desirable to determine the effect of Bios on the growth of plant roots after the plants had become established in the absence of Bios. For this purpose, a layer of Crone's agar medium containing Bios 2 enrichment in optimum concentration was placed in the bottom of a quart jar, and another layer, without Bios addition, was superimposed on this. Seeding was carried out as previously described. The seedlings grew normally, and when, after one week, the roots reached the layer containing Bios, they neither turned up nor did they show any other observable effect from the Bios concentration. It would seem probable, under certain conditions at least, that the factor which causes such marked changes in the hypocotyl as observed in the primary stages of growth, does not exercise any influence on the root tissue once the early seedling stage has passed.

Although, when tested separately on bacteria, no distinct difference had been observed in the action of Bios 2(a) and Bios 2(b), it was considered possible that one or the other of these factors might be specifically responsible for the "root bending" phenomenon. Experimental results showed that an agar seed bed enriched with Bios 2(b) alone brought about bending of the hypocotyls, while the addition of Bios 2(a) alone exercised only a very slight influence, if any, in this respect. Crude Bios 2(a) and 2(b) preparations obtained after the manner of Miller et al. (9), were likewise tested and gave similar results, though crude Bios 2(b) was not as active in producing "root bending" as the more refined Bios 2(b). This finding lends support to the

conception of Miller that Bios 2 is of a multiple nature, and it further indicates that the new fractionation method results in a reasonably complete separation of two distinct entities as represented by Bios 2(a) and 2(b) respectively.

Recently Miller (10), made the suggestion that the active constituents of Bios 2(a) might be  $\beta$ -alanine and l-leucine. These amino acids were included in the tests along with aspartic and glutamic acids, arginine, cystine, tyrosine, tryptophane, and histidine; none of these compounds caused bending of the hypocotyls. Negative results were also obtained with Bios 1 (inosite). Similarly, the pantothenic acid of Williams which has been reported to stimulate the growth of alfalfa (8), and Riccia (13), did not possess the effect of Bios 2(b) as shown on red clover plants. Various compounds, carnosine, indole, scatole, guanine, ergothioneine, and glutathione were also found to be inactive.

In order to determine whether or not the "root bending" phenomenon might be due to the toxicity of the heavy metals used, and possibly carried through in small quantities into the Bios preparation, these metals (barium, mercury, and lead) were added to the medium in minute amounts. General evidence of toxicity was observed, but otherwise the metals caused no change

in the normal root development.

In the light of the work of Kogl (6, 7), and Went (12) regarding the effect of auxins on plant growth in general, it was thought desirable to enquire whether or not a corresponding "root bending" phenomenon might be caused by these substances. A priori it was realized that the treatment of auxins with heat, in the presence of dilute acid and alkali respectively, results in their destruction, while Bios 2(b) is quite stable under similar conditions. Besides, auxins are soluble in ether, and Bios 2(b) is not. Thus on the basis of chemical properties it did not seem probable that any of the known auxins could be responsible for the "root bending" of the type referred to above. However, the response of red clover seedlings to hetero-auxin ( $\beta$ -indolyl acetic acid) was studied in a manner similar to that already described for Bios, except that while the latter was used in a 2\% concentration, the hetero-auxin, after being tested at six different concentrations ranging from 0.0001 to 0.01 mg. per cc., was used at a concentration of 0.0005 mg. per cc. Germination of the seeds revealed, that while hetero-auxin, like Bios 2(b), does not permit the hypocotyls to enter the substratum, there are, at the same time, apparent differences in the action of the two agents on red clover seedlings. As previously reported by other workers (12), hetero-auxin causes the development of thick, heavily swollen hypocotyls not observed in the case of Bios 2(b). The latter, again, compels the hypocotyls to extend upwards from the cotyledons, which remain resting on the seed bed, while hetero-auxin, although not permitting the hypocotyls to enter the seed bed, causes a slight horizontal twisting of the hypocotyls and allows the cotyledons to occupy their normal upright position. Finally, Bios 2(b) inhibits the formation of root hairs on the primary roots, while hetero-auxin has no such inhibitory influence. Hetero-auxin and Bios 2(b) may, therefore, be distinguished on the basis of either their chemical properties or their physiological effects on the development of red clover seedlings.

After having demonstrated the dissimilarities between the auxins and Bios 2(b), the attention of the writers was called to the recent work of Bonner (1) who, in seeking an ideal medium for plant tissue cultures, found that additions of plant extract were essential to the successful growth of his cultures. His statement regarding the properties of the active compound in the extracts employed indicates a close relationship to Bios 2 fraction, and his reference to the adsorption of the active substances on charcoal would suggest that the compound with which he was concerned might be identical with Bios 2(b). By applying a drop of his extract to the parenchymous lining of the cup-like depressions inside a bean pod, Bonner reports the production of a "wart-like protuberance". A similar effect was observed following the pricking of such cells with a pin. The auxins, vitamins B<sub>1</sub> and B<sub>2</sub> and pantothenic acid were shown by him to be inactive in this respect.

On the assumption that Bios 2(b) might be the causative factor, the writers conducted a similar test using this activator, with hetero-auxin, water, and pin-pricks as controls. Bios 2(b) produced protuberances which appeared to be similar in all respects to those described by Bonner, while hetero-auxin and water were ineffective. Histological preparations showed that an intensive cell multiplication occurred from the addition of Bios 2(b) where Phaseolus vulgaris was tested, while in the case of Phaseolus coccineus, cell elongation was chiefly responsible for the swelling. Pin-prick controls gave evidence of a small amount of cell multiplication. These results would appear to strengthen the hypothesis advanced by Went in which Bios was assigned the properties of a "wound hormone".

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The authors wish to express their thanks to Professor P. A. Boving for much helpful criticism in the preparation of the manuscript; to Dr. R. J. Williams of Oregon State College for  $\beta$ -alanine and pantothenic acid, and to Dr. B. A. Eagles for many of the remaining compounds used in this study.

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# ACTIVITY OF THE VASCULAR CAMBIUM IN RELATION TO WOUNDING IN THE BALSAM POPLAR, POPULUS BALSAMIFERA L.<sup>1</sup>

By A. B. Brown<sup>2</sup>

#### Abstract

An investigation of the response of the vascular cambium to wounding in leader shoots of the balsam poplar, Populus balsamifera L., was carried out in the laboratory during the winter months, at which time observations on local wound cambial activity, distinct and apart from normal cambial activity, can be obtained. It was found, in disbudded units, that the greater the amount of living bark distal to a wound, the greater is the development of local cambial activity in relation to the wound. Local wound cambial activity is further promoted by the presence of developing buds and leaves distal to the wound, and the effect can be observed before the basipetal gradient of normal cambial activity emanating from the developing extension growth has reached the wound. Cambial activity in relation to wounding responds to gravity in the same way as normal cambial activity. In horizontally placed leader shoots, cambial activity is greater in relation to a wound on the upper side of the shoot than to a similar wound directly opposite on the lower side. On the basis of these results, it is suggested that a hormone, present in the living bark and also produced by developing buds and leaves, is involved in local wound cambial activity. In all probability this hormone is identical with that which promotes normal cambial activity.

It is also suggested that a wound substance, capable of promoting by itself cell division only, is involved in local wound cambial activity. The amount of this wound substance produced is apparently proportional to the extent of dying of the cells of the bark subsequent to wounding. From the lower edge of a complete ring, a very feeble basipetal gradient of cambial activity arises, in which differentiation to form vessels and fibres does not occur, although a few tracheids may be found. This type of behavior has not hitherto been reported, and is interpreted as the result of stimulation of the cambial layers by the wound substance alone. Local cambial activity above a complete ring and in relation to bridged wounds, involves differentiation of more or less typical vessels and fibres, and is interpreted as the result of interaction between the wound substance and the cambial hormone traveling basipetally in the living cells of the bark. The absence or feebler development of cambial activity at certain points in relation to bridged wounds, in contrast to greater development at other points where presumably the concentration of cambial hormone must be less, is interpreted as the result of lack of wound substance or low concentration of it acting as a limiting factor.

#### Introduction

For a long time it was thought, and apparently still is by many, that the vascular cambium plays an important role in the formation of callus tissue, but this belief derives no support at all from the recent work of Sharples and Gunnery (19), and of Sass (18). According to these investigators, callus tissue arises as the result of proliferation of medullary ray cells particularly, and not from the vascular cambium generally. However it is known definitely that the cambium responds to wounding, under certain conditions, to produce vascular elements.

Hartig (9) was the first to observe the basifugal development of cambial activity from the upper edge of a complete ring in woody shoots, and Sledge (20) has greatly increased our knowledge of this phenomenon in recent years.

Manuscript received October 31, 1936.
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This upward spread is of limited extent, and is quite independent of the presence of growing buds and leaves, upon which the normal basipetal development of cambial activity in the shoot does depend. The writer (2) has also observed the same type of thing in ringed roots of poplar. Swarbrick (24) mentions an interesting case of local cambial activity as a result of wounding, in a completely disbudded apple shoot. In one of his experiments a small triangular piece of cortex and phloem was accidentally torn out, so as to leave the xylem exposed. Upon examination some time later, cambial activity was observed above, below, and lateral to the wound.

A major communication on the subject of tissue reorientations in woody plants, in response to wounding, has been contributed by Janse (10). He conducted an extensive series of ringing and wounding experiments, and appears to have been the only investigator to pay any attention to the behavior obtaining below bridged rings and other types of wounds. The main value of his publication lies in his observations as such and the abundance of excellent photographs illustrating them, rather than in his interpretation of his results. Apparently, his experiments were carried out during the growth period when normal cambial activity was proceeding, and he has nothing to say about local wound cambial activity independent of normal cambial activity. He showed, among other things, that when an oblique bridge of bark was left connecting the upper and lower edges of a ring, the cells within the bridge soon became reoriented to run in the same direction as the bridge, and that this change was a gradual one, becoming more and more marked in each successive layer of cells cut off by the cambium. A little later Teodoresco and Popesco (25) studied callus formation and the reaction of the cambium in relation to a ring in which a bridge of bark in the form of a step (Czapek's ring) was left, connecting the upper with the lower edge. The wound was made when the cambium was active, and they found that ultimately the elements in the horizontal part of the bridge became reoriented to run transversely, with the result that uninterrupted vascular continuity between the parts above and below the wound was re-established. This type of behavior is also discussed by Priestley (15) in relation to his theory of symplastic growth, and a detailed investigation of the changes involved in the reorientation of the cambium within the bridge has been carried out by Tupper-Carey (27). Somewhat similar results, although less detailed, are reported by Collins (4), who found that he could obtain zig-zag and spiral grain of the wood as a result of appropriate wounding.

This present investigation involves a critical study of the reaction of the vascular cambium to different types of wounds made in shoots during the winter period. In this way it was possible to study local wound cambial activity for a considerable period of time before the normal basipetal flow of cambial activity from developing extension growth had reached the wound, and in completely disbudded units, apart altogether from normal cambial activity. A number of significant correlations have revealed themselves, on the basis of which the response of the vascular cambium to wounding is

interpreted in terms of the interaction between a hormone present in the living bark, which is very probably identical with that promoting normal cambial activity as described recently by Snow (23), and a wound substance produced subsequent to wounding.

#### Material

The material used throughout this investigation was *Populus balsamifera* L., the balsam poplar. Main stems or leaders only were employed, and some 600 trees, yielding more than 1,000 experimental units, have been involved in the work. The units practically always consisted of a length of shoot derived from the extension growth of one particular year, and varied in age from one to four years. All the experiments were done in the laboratory during the winter months, from December to May, during which time the cambium was dormant in material in the field. The wounds were smeared with vaseline and the shoot placed vertically with its basal end immersed in water, where rooting took place very readily as the result of rapid development of preformed root initials. Normal bud-break took place in the laboratory, and leaves began to appear about two weeks after the units had been set up in water. In all the experiments to follow the shoot was placed vertically, unless otherwise stated.

It was found that by peeling the bark from the shoots, at the end of the experimental period, and allowing them to dry out, the new xylem formed locally subsequent to wounding showed up clearly upon the surface of the old wood. This procedure was used extensively throughout the investigation and proved to be of great value.

#### **Oualitative Experiments**

#### (a) Activity Above and Below a Complete Ring

Cambial activity immediately above a complete ring in balsam poplar is essentially similar to that already observed by Hartig (9) and Sledge (20) in other woody plants. It is a local development, independent of normal cambial activity emanating from developing extension growth, and is expressed by a very obvious basifugal gradient of xylem formation, spreading for a short distance distally from the upper edge of the ring. There is a marked tendency for this new xylem to be "piled up" just above the ring. In appearance this wood is not markedly atypical, at all events in transverse section. It consists of vessels, tracheids and well thickened fibres, with nests of parenchyma not infrequently included. The most obvious departure from typical stem wood is to be found in the shape of many of the vessel segments. They are narrow, and might readily be identified in longitudinal sections as tracheids with long tapering ends. On maceration of the wood, however, these elements reveal themselves as vessel segments with very oblique end walls which are for the most part pitted, and the perforations between these vessel segments occur laterally at maturity, whatever their place of origin may have been morphologically.

The general belief is that no cambial activity originates from the lower edge of a complete ring, but this is certainly not the case in balsam poplar, where it was found that a very feeble basipetal gradient of cambial activity does develop from the lower edge of a complete ring. Apparently the cambium cuts off cells mostly to the inside. In transverse section (Plate I, Fig. 3) these cells are more or less uniformly rectangular in shape. They remain thin walled and unlignified with the exception of occasional small groups which lignify, without appreciable expansion in any direction, to form scalariform and reticulately pitted tracheids. Vessels and fibres have not been found to occur. As many as 10 to 15 rows of cells, cut off to the inside in this manner by a well defined cambium, have been observed just below the lower edge of a complete ring. The gradient falls off rapidly, and usually within the space of a few millimetres ceases to be easily detectable.

It has, however, been observed clearly in many cases for a distance of 1 cmfrom the lower edge of the ring. Relative to that obtaining just above a complete ring, the amount of cambial activity developing basipetally from the lower edge is indeed small. As far as the writer is aware this type of development has not been reported before. Sledge (20), in one case only, an internodal cutting of *Sambucus*, detected cambial activity at the distal

> end, where a few new elements had been cut off at one place, but apparently not completely around the shoot as in poplar.

> A simple modification (Fig. 1) of the complete ring yielded results of marked significance. In this case the ring was very wide, and a tongue of bark (cortex and phloem) was left, running distally from the lower edge. After some weeks the material was examined for cambial activity, with the result expressed by the dotted lines in the diagram. A feeble basipetal development of cambial activity was detected spreading downwards from the transverse margins, but extremely little laterally from the longitudinal edges of the wound. The elements formed were of the type already described as occurring below an ordinary complete ring, i.e., unlignified, with the exception of a few tracheids. A correlation that may be of some significance in this connection is that dying subsequent to wounding is always more extensive longitudinally from the transverse edges of the wound, than it is laterally from the longitudinal edges.

No evidence was obtained that cambial activity from the lower edge of a complete ring is dependent upon the development of adventitious buds, which occasionally arise from the callus tissue in this

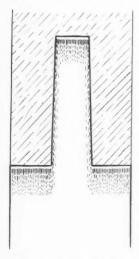


FIG. 1. Illustrating greater development of cambial activity longitudinally from the transverse margins than laterally from the longitudinal margins, in a wound where a longitudinal tongue of bark runs distally up the shoot, from what is really the lower edge of a complete ring.

region. Such buds promote a local obliquely basipetal development of cambial activity in the shoot below them, but this activity is easily distinguished from the uniform development that is to be observed when no adventitious buds are present. Moreover there was no question of any stimulus to cambial activity passing across the ring, for precisely the same type of behavior can be observed at the distal end of completely disbudded cuttings. The foregoing results are also of interest when compared with callus formation in relation to ringing. Callus formation, just as cambial activity, is more marked at the upper edge of a complete ring than it is at the lower, where it does however usually occur to some extent.

## (b) The Longitudinal Bridge

The following observations apply to local cambial activity in relation to a wound where, instead of making a complete ring, a longitudinal bridge of bark is left, connecting the parts above and below the wound. In some shoots a complete ring was made some distance above the wound under consideration, and the shoot completely disbudded and lateral branches removed between the complete ring and the wound below. In others no complete ring was made above the wound, but the shoot was disbudded and laterals removed for a considerable distance above the wound. This was done in order to make it possible to observe local wound activity for some considerable time before the normal basipetal development of cambial activity

from the developing extension growth reached that point. Actually however the sequence of events was exactly the same in both groups. Fig. 2 and Plate 1, Fig. 1 illustrate the result obtained in relation to a wound of this type after two to four weeks. The arrangement depicted is visible to the naked eye when the bark is peeled off and the shoots allowed to dry out. It will be observed that just distal to the upper edge of the wound, a well defined basifugal gradient of xylem has been laid down, essentially similar to that obtaining above a complete ring. Within the bridge, however, cambial activity is basipetal, being undoubtedly more evident at the distal end of the bridge during the earlier stages of development. In transverse sections through the bridge it will be found that wedges of new wood, tapering off to the inside from the edges of the bridge, have been formed. Below the bridge, cambial activity swings round on both sides to run obliquely down and around the shoot. The first signs of cambial activity are always to be observed just distal to the upper edge of the wound. Notice (Fig. 2) that there is no cambial activity in the median line of

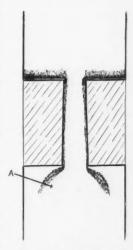


FIG. 2. Local cambial activity in relation to a longitudinally bridged ring, expressed in terms of xylem formation as revealed when the bark is peeled from the shoot.

the bridge itself, or in the shoot above and below in the same longitudinal line as the bridge. Particular attention should be paid to the fact that there is no cambial activity in the region A (which is definitely lateral to the longitudinal bridge), whereas cambial activity does occur lateral to it, not only at the same transverse level but also below that level. The obliquely basipetal spread below the wound runs for a greater distance down the shoot than the basifugal development above the wound extends up the shoot. However, the depth of wood radially is always greater just above the upper edge of the wound than at any other point. Another especially interesting feature is that there is definitely more cambial activity (including cell divisions, vessel formation and lignification) below the wound than within the bridge itself. This is very different from the state of affairs obtaining in relation to a similar wound coming under the influence of the normal basipetal development of cambial activity for at least some considerable time. The writer (2) has observed in aspen poplar, that in such a wound either in the shoot or the root, the width of the growth ring is always greater within the longitudinal bridge than it is above or below. Teodoresco and Popesco (25) also make a statement to the effect that the amount of new xylem and phloem formed within the

zig-zag bridge in their experiments was abnormal, as if an attempt were being made to replace the tissues which had been cut away.

Development has been followed for as long as eight weeks, but no significant changes occur. There is a general increase in the amount of cambial activity (Fig. 3). The wedges of xylem within the bridge ultimately meet, provided the bridge is not too wide and, again depending upon the width of the bridge, the depth of wood may become more or less uniform across the bridge. Coincident with this, new xylem may make its appearance to a greater or less extent, above and below the wound, in the same longitudinal line as the bridge. The obliquely basipetal development below the wound becomes more extensive, and swings round on both sides to meet behind. At the end of eight weeks, in those shoots with no complete ring above the wound, the normal basipetal development of cambial activity from the buds and leaves had usually reached the wound, but even then, in this material, there was still less cambial activity within the bridge, relative to that obtaining below the wound.

The xylem developing basifugally from the upper margin of the wound is essentially similar to that above a complete ring, consisting of vessels

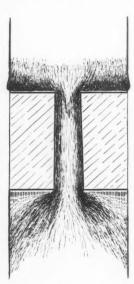


Fig. 3. Same as Fig. 2, but showing more extensive development. The dotted lines indicate feeble cambial activity similar to that occurring below a complete ring, in which there is no differentiation of vessels or fibres.

(mostly atypical and of the type already described), tracheids, fibres and included groups of parenchyma. Within the bridge the new wood is usually more or less normal, except for the not infrequent presence of parenchyma. The obliquely basipetal development below the wound is, however, particularly interesting. Actually in that sheet of tissue the cells are all oriented. at least for a very considerable period of time, in the normal longitudinal direction. Undoubtedly development is obliquely basipetal, but it would not be so obvious to the naked eye perhaps, were it not for the peculiar mode of development of the vessels. In this new wood, vessels do not arise from the progressive vacuolation of vessel segments in longitudinal series, but rather from a series of cells running obliquely down and around the shoot. The result is that it is only the vessels as a whole that run obliquely basipetally, whereas the vessel segments themselves, the fibres, the unlignified parenchyma and the medullary rays practically retain their normal orientation (Plate I, Fig. 5). The vessel segments are atypical, however, in that the end walls are definitely more oblique than usual, and the perforations between vessel segments tend to be lateral at maturity. Moreover the vessel segments are often shorter than those of the typical vessel. Even after as long as eight weeks, very little progress in the direction of a general reorientation of the tissue elements was observed in this material. A general reorientation is however complete within a much shorter period of time below a similar wound in the normally growing tree, during the season of active growth. These observations would seem to indicate that the process of vessel formation plays rather an important part in tissue orientations of the type just described.

There still remains for consideration one important point in connection with the longitudinally bridged wound. In that area below the wound which is missed by the strong obliquely basipetal spread, one can detect a very feeble basipetal development of cambial activity from the lower transverse margin of the wound. This development, which is by no means obvious to the naked eye, is represented in Fig. 3 by the dotted lines, and is exactly the same as that arising from the lower margin of a complete ring (c.f. Fig. 1), in which vessels and fibres do not differentiate (c.f. Plate I, Fig. 3). Actually this basipetal development can be detected most readily in that region of the shoot, behind and below the longitudinal bridge, where the distance between the lower transverse margin of the wound and the strong obliquely basipetal spread is greatest. Within the bridge itself, the exceedingly feeble stimulation of cambial activity laterally from the longitudinal margins, corresponding to that obtaining in a distally running tongue of bark, as previously described (Fig. 1), is completely masked by the stronger basipetal development down the bridge.

It may be stressed at this time, that a similar feeble basipetal development of cambial activity, in which vessels and fibres do not differentiate, can be detected below all bridged wounds, in that region missed by the strong obliquely basipetal spread which is characterized by differentiation of vessels and fibres. For simplicity, however, this development will not be depicted in the drawings to follow, in which only the obvious cambial activity, expressed in terms of xylem formation, involving differentiation of vessels and fibres, will be illustrated.

Fig. 4 illustrates cambial activity in relation to another wound which is really a modification of the type just considered. In this case a rectangular piece of bark has been cut out. It is hoped that the drawing

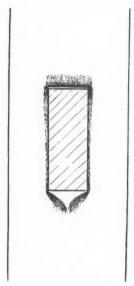


Fig. 4 Local cambial activity in relation to a rectangular wound, expressed in terms of xylem formation.

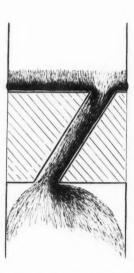


Fig. 5. Local cambial activity in relation to an obliquely bridged ring, expressed in terms of xylem formation.

more or less explains itself and nothing more will be said regarding it. An essentially similar arrangement is obtained around the wound resulting from the removal of a circular piece of bark.

#### (c) The Oblique Bridge

A series of experiments was also set up, in which an oblique or spiral bridge of bark, instead of a longitudinal one, was left connecting the upper and lower edges of the wound (Fig. 5). It is proposed to allow the diagram to tell its own story more or less, which permits the following descriptive remarks to be brief. Here again a basifugal gradient of xylem is laid down from the upper edge of the wound. Development below the wound is obliquely basipetal, just as in the previous experiments. Within the bridge itself development is also obliquely basipetal, the grain of the wood running parallel to the edges of the bridge. The following significant points should be noted. Within the bridge cambial activity is most marked at the lower edge and

decreases towards the upper. The wider the bridge the more obvious this is, but in a very narrow bridge it may not be detected at all. The lateral spread in the shoot below the wound is always more extensive on that side below the acute angle formed by the lower edge of the wound and the bridge. In the bridge itself reorientation of all the elements, including the medullary rays, to run in the same direction as the bridge, takes place rather quickly, and may be complete at the end of five weeks. Below the bridge, however, reorientation is much slower, and at the end of eight weeks is still more or less confined to the vessels as a whole, the vessel segments as individual units and all the other elements still running practically longitudinally, in the same manner that obtains below the wound with a longitudinal bridge. A similar state of affairs is to be found in the bridge itself during the earlier stages of development (two to three weeks). In sections through the bridge and transverse to the shoot, in material that has been developing for a longer period of time (five to eight weeks), it is found that the elements of the firstformed xylem are all cut transversely and that the later-formed elements are cut progressively more and more obliquely until reorientation is complete. Here again the first expression of reorientation is to be found in the process of vessel formation from a number of segments in obliquely basipetal series. In contrast with the longitudinal bridge it is not infrequently found that there is a greater radial width of new wood within the oblique bridge than occurs at any point in the shoot below it, particularly if the bridge is rather narrow.

## (d) The Zig-zag Bridge (Czapek's ring)

Development at an early stage, 10-14 days, in relation to this type of wound is depicted in Fig. 6. The first signs of cambial activity may be observed about the same time, just above the upper margin of the wound and in the horizontal part of the bridge exclusive of that area which is common to the lower vertical strip. Within the upper vertical strip cambial activity is basipetal and essentially similar to that in the simple longitudinal bridge. Notice that an obliquely basipetal development from the base of the upper vertical part of the bridge into the horizontal portion is shown. This may not be obvious if the bridge is very narrow. In the lower vertical strip, cambial activity is for the most part basipetal, but towards the distal end there is also a definite obliquely basipetal spread across the bridge from the horizontal strip to the farther margin of the vertical strip. Below the wound an obliquely basipetal development on both sides is again found, and during this earlier stage development is often rather more marked on

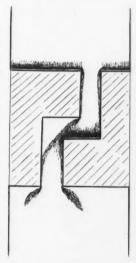


Fig. 6. Local cambial activity in relation to Czapek's ring (zig-zag bridge) expressed in terms of xylem formation.

one side, viz., that side directly below the horizontal portion of the bridge. Otherwise the situation below the wound is exactly the same as that obtaining below the simple longitudinal bridge, and again it is usual to find relatively more cambial activity in the obliquely basipetal spread below the wound than occurs in the basipetal development down the vertical portions of the bridge.

A later stage in development, after four or five weeks, is shown in Fig. 7. There is a marked "piling up" of new xylem, not only above the upper margin of the wound, but also in the horizontal part of the bridge. Usually there is no appreciable difference in the amount of cambial activity in the upper and lower vertical strips. Provided the bridge is fairly narrow, the obliquely basipetal development below the wound may ultimately be practically equal on both sides, but if the bridge is sufficiently wide the greater spread on one side may be maintained.

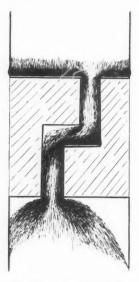


Fig. 7. Same as Fig. 6, but showing more extensive development.

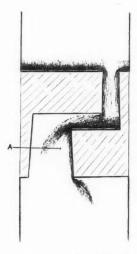


FIG. 8. Local cambial activity in relation to a simple modification of Caspek's ring, in which the lower vertical strip of the zig-zag bridge is made quite wide.

Fig. 8 shows a simple modification of the zig-zag bridge, in which the lower vertical strip is very wide. In such a wound the obliquely basipetal spread of cambial activity from the horizontal strip across the lower vertical strip, and the basipetal spread down the nearer longitudinal margin stand out as two distinct developments, diverging from one another so as to leave an area (A) in which there is no cambial activity. The diagram was drawn from a specimen, three weeks after the beginning of the experiment. After a longer period of time the obliquely basipetal development may ultimately

reach the farther margin of the lower vertical strip and travel basipetally down it, just as in the narrower zig-zag bridge depicted in Fig. 6. The phenomenon just described is of great significance and will be enlarged upon later.

The structural characteristics of the basifugal development of xylem above the wound, of the basipetal development in the two vertical strips and of the obliquely basipetal spread below the wound are exactly similar to those obtaining in the same relative positions in the wound with the longitudinal bridge of bark, and no further comment is called for in this connection. Within the horizontal strip of the bridge, all the elements of the first-formed xylem retain their normal orientation in the longitudinal direction, but the vessels arise from a number of segments in lateral or obliquely lateral series. As development proceeds, the situation becomes quite complex within the horizontal strip, involving much twisting and turning of the elements, and resulting in a very complicated grain of the wood. Even after as long as eight weeks, complete and uniform reorientation of the elements in the transverse direction was not observed, in this material, but it does occur ultimately in a similar wound in the actively growing tree.

Tupper-Carey (27) has followed in detail the structural changes involved in reorientation of the tissues within the bridge in Czapek's ring. Her conclusions need not be discussed here, but she does not stress at all the process of vessel formation as an important factor in this reorientation. In view of the observations, made in this present investigation, on the process of vessel formation from a number of cells in obliquely basipetal series (Plate I, Fig. 5), within the oblique bridge and below all three bridged wounds, the writer is strongly inclined to attribute to this process an important role in the ultimate reorientation of the tissues as a whole, and is also of the opinion that the details might be studied more conveniently, and certainly with less complications, in the oblique bridge than in the zig-zag bridge. These observations lend some measure of support to a former suggestion by the writer (1), that reorientation of the tissues in the form of a flow-pattern, below the sucker bud in poplar roots, may be linked up with the process of vessel formation.

#### Quantitative Experiments

A large number of shoots were treated as follows. Towards the distal end a complete ring was made, and all buds and lateral branches below the ring removed. Some distance below the ring, for example two inches, a definite wound was made, and then another complete ring at, let us say, four inches below that wound. Finally another wound, exactly similar in shape and size to that between the two complete rings, was made at a much greater distance, for example ten inches, below the second complete ring. The shoot was cut through four inches below this wound and then set up vertically with its basal end immersed in about one inch of water. The definite wounds referred to might be of any type. Actually a circular wound made by means of a sharp cork borer, and bridged rings were used extensively.

The exposed xylem was thoroughly scraped to ensure destruction of the cambium, and the wounds were vaselined in the usual way. The distances between the wounds and the complete rings above varied according to the material, but the situation was always this, that the shoot bore two similar wounds, one of which had a short length of bark distal to it, and the other a much longer length of bark distal to it. In some the shorter unit was distal on the shoot, and in others the longer. On examination after four or five weeks it was invariably found that there was more cambial activity (new xylem) around the wound with the greater length of bark distal to it.

The same relation between the amount of cambial activity around a wound and the amount of bark distal to the wound was also expressed clearly and invariably in another type of experiment (Plate I, Fig. 1). In the particular experiment illustrated the arrangement was as follows. Unit C had a complete cylinder of bark, seven inches in length, distal to the longitudinal bridge. Unit B had the same length of bark distal to the bridge, but the area of bark was much reduced by removal of about half the cylinder, and in the third unit A about three-quarters the cylinder of bark was removed, so that only about one-quarter remained in the form of a long narrow strip seven inches in length. The three units were all located on the same shoot in three-year-old wood, and within the limits of a single year's extension growth. Unit A was the most distally situated on the shoot, and unit C was distal to unit B. In other similar experiments the relative positions of these units to one another were varied. The result of such an experiment is expressed clearly in the photograph (Plate I, Fig. 1), which shows the extent of xylem formation, as revealed when the bark was stripped off after 4½ weeks. Here again it is evident that the more bark there is distal to a wound, the greater is the extent of development of cambial activity in relation to the wound. It does not follow that the width of the transverse cut as such influences in any way the amount of cambial activity, but it does indicate lateral transportation of the stimulus to cambial activity.

Still another experiment, leading to the same conclusions with regard to the proportional relation between wound cambial activity and the amount of bark distal to the wound, and also to lateral translocation of the stimulus to cambial activity, is depicted in Fig. 9. Here a spiral tongue of bark was left running distally from the lower margin of a very wide ring. It was observed that xylem formation increased gradually towards the base of the spiral tongue.

Experiments were also performed to determine the effect of developing buds and leaves within the unit. Main shoots of the same age were selected in pairs, the members of each pair being as uniform in all respects as could be determined by external examination. In one of each pair, a definite wound was made, and the shoot completely ringed some considerable distance, at least 12 inches, distal to the wound. The unit was completely disbudded below the complete ring. The other member of the pair was cut so that approximately the same amount of bark was left distal to the wound but a

few buds, usually two or three, were allowed to remain and develop towards the distal end. Only the distally situated buds were left, because it was desired to observe their effect upon wound cambial activity before the normal basipetal gradient of cambial activity from the developing buds and leaves had reached the level of the wound. The experimental units were therefore of two types, those bearing buds and those without buds. The shoots composing the latter did of course bear buds above the complete ring distal to the wound, but these buds were without the experimental unit as such. Many types of wound were employed, but for microscopic examination the longitudinally bridged wound is perhaps the most convenient.

The leaves began to emerge from the buds after about two weeks, and the material was examined at intervals later. In this way definite evidence was obtained, in several series of experiments, that the presence of developing buds and leaves within the unit increased the amount of cambial activity in relation to the wound, and this effect was observed undoubtedly before the normal basipetal gradient of cambial activity emanating from the developing extension growth had reached the wound. The clearest results were obtained four to six weeks after the beginning of the experiment, i.e., two to four weeks after emergence of the leaves from the buds. Not only was there a definite increase in the amount of cambial activity around the wound, but lignification of the new xylem was also more marked. There was also a tendency, not invariable, however, for the vessels to be wider when developing buds and leaves were present on the unit. The above effects were not observed until after the buds had opened and the leaves had developed to a considerable extent, which would indicate that the increase in wound cambial activity is to be related not simply to the presence of buds within the unit, but rather to the development of leaves from these buds.

Table I, showing the number of vessels at certain points in relation to a wound in 14 pairs of contrasting units, with and without developing buds and leaves, gives some idea of the results obtained. In the first four pairs, a small rectangular piece of bark was cut out on March 30, the length of

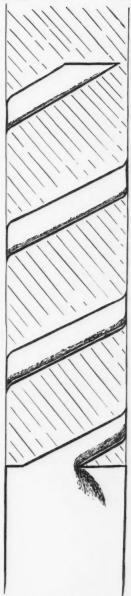


Fig. 9. Cambial activity, expressed in terms of xylem formation, in relation to a spiral tongue of bark running distally up the shoot from a transverse margin.

bark distal to the wound was 12 inches in all cases, and the experiment ran until May 2. The number of vessels that had developed down the longitudinal margins of the wounds was counted from transverse sections. In the next six pairs of units, the wound employed was Czapek's ring, there

TABLE I
Numbers of vessels in contrasting pairs of units, with
and without developing buds
and leaves

Without buds	With buds
42	87
40	76
25	98
26	53
69	104
80	144
28	65
154	234
67	113
88	217
101	265
173	264
178	324
231	327

were 12 inches of bark above the wound in all cases, and the experiment ran from March 30 until May 3. The figures given are the numbers of vessels in transverse sections of the lower vertical strip of the bridge. In the last four pairs a circular wound was employed, there were 20 inches of bark distal to the wounds, and the experiment ran from April 6 until May 7. The vessels were counted in sections cut through the diameter of the wound and transverse to the unit.

In none of the material considered in the foregoing table had the normal basipetal gradient of cambial activity reached the wound in units bearing developing buds and leaves.

The effect of developing buds and leaves is illustrated quite typically, in two photomicrographs (Plate I, Figs. 4 and 6) of sections from a contrasting pair of units in another experiment. In this case the wound employed was

the longitudinally bridged ring. There were 19 inches of bark distal to the wound in both units, and the experiment ran from April 18 until May 27. The sections photographed were obtained from corresponding positions within the longitudinal bridge in both units. Plate I, Fig. 4 shows the extent of development in the unit in which the two most distally situated buds were allowed to develop, and Plate I, Fig. 6 illustrates the corresponding development in the completely disbudded unit. In this particular case the normal basipetal gradient of cambial activity faded out about three inches above the wound, in the unit bearing developing extension growth.

Sledge (20) reports that he found no difference in the amount of cambial activity at the base of disbudded cuttings relative to control cuttings, after a period of one month. The present writer found the complete ring a very unsatisfactory type of wound to use in quantitative experiments, on account of the "piling up" of cambial activity above the ring, which made it extremely difficult to detect any but the most obvious differences in the extent of development, even when the material was examined microscopically. A similar "piling up" of cambial activity also takes place at the base of woody cuttings, where the conditions are essentially similar to those above a complete ring.

## The Effect of Gravity upon Wound Cambial Activity

Experimental units were completely ringed towards the distal end, and all buds and laterals below the ring removed. Some considerable distance, at least 12 inches, below this ring a modified ring was made, in which two longitudinal bridges on opposite sides of the shoot were left connecting the proximal and distal margins. Another complete ring was then made about six inches below this wound, and the shoot cut through approximately six inches below this second complete ring. In other units, two circular wounds on opposite sides of the shoot were often employed instead of the doubly bridged ring. The shoots were arranged practically horizontally with their basal end in water in a very simple manner, viz., by inserting them into ordinary cylindrical metal cans, about five inches in diameter and with an inwardly projecting rim, so that the basal end lay under the rim, and the shoot rested upon the opposite edge. If the cans were filled with water, two shoots pointing in opposite directions could be conveniently inserted in this manner, and a very stable arrangement resulted. The units were arranged so that the longitudinal bridges, or the circular wounds, lay one directly above the other, on opposite sides of the shoot.

After about one month, material that had been kept in complete darkness during the period of the experiment, was examined for local wound cambial activity, when a marked response to gravity was clearly revealed. There was always distinctly more cambial activity on the upper than on the lower side. This response was evident not only in relation to the doubly bridged wound and the circular wounds, but also just distal to the lower complete ring. It was also observed that in many cases the preformed root initials on the upper side had undergone marked swelling, where they occurred along practically the full length of the unit, whereas no such activity was evident on the lower side. Plate I, Fig. 2 shows clearly, in longitudinal and transverse section, the marked difference in the amount of new xylem on the upper and lower side, immediately distal to the lower complete ring in two three-year-old units which had been developing in the horizontal position from May 13 until June 10, in complete darkness. The same marked response was obtained in material developing horizontally in diffuse daylight where the upper side received more light than the lower, but the response is clearly one to gravity and not to light. Actually a number of experiments, which need not be described here, were performed to test the action of diffuse daylight, and no evidence of a light effect on the amount of wound cambial activity was obtained. The effect of gravity was still quite marked in units lying at an angle of 45° to the horizontal.

In another experiment, horizontally placed shoots were rotated through an angle of 180° once every 24 hours for one month, and at the end of that time no difference in the amount of wound cambial activity on opposite sides of the unit could be detected.

Similar experiments were also set up to determine the effect of gravity on the normal basipetal development of cambial activity emanating from growing. buds and leaves. In this case lengths of main shoot were trimmed so that only the more distal buds remained. They were placed horizontally with their basal end in water in the manner previously described, and examined about two months later, when it was found that there was distinctly more cambial development on the upper than on the lower side. Priestley and Tong (16) obtained the same response to gravity in vertical shoots of *Acer* and *Fraxinus*, after they had caused them to develop in the horizontal position for some time. Apparently, therefore, normal cambial activity and local wound cambial activity in balsam poplar respond similarly to the force of gravity. No difference in the degree of lignification of the xylem on the upper and lower sides in horizontally placed units was detected, either with respect to normal cambial activity or to local wound cambial activity.

It will be recalled that a very feeble basipetal gradient of cambial activity, in which vessels and fibres do not occur, arises from the lower margin of a complete ring, but no difference in the extent of development on the upper and lower side in horizontally placed units could be detected.

# Discussion and Interpretation of Results

#### (a) Quantitative Results

Snow (23) has very recently succeeded in promoting apparently normal cambial activity in shoots by means of pure auxin-a and hetero-auxin  $(\beta$ -indolyl acetic acid), thus confirming his earlier conclusion (22), that the stimulus emanating from the developing leaves to activate the cambium in the stem below is of the nature of a hormone. In the opinion of the present writer, hormone action appears to supply the most reasonable explanation of the quantitative results obtained in the foregoing experiments on cambial activity in relation to wounding. This suggestion is based upon the following considerations. A definite quantitative relation has been established between the amount of cambial activity in the vicinity of a wound and the amount of bark distal to the wound. This result would be readily understood in terms of the downward movement of a hormone present in the bark. It has also been shown that wound cambial activity is further promoted by the presence of developing leaves distal to the wound, an effect which can be detected before the normal basipetal development of cambial activity emanating from these leaves has reached the wound. This indicates that developing leaves are a source of the hormone promoting wound cambial activity, which suggests further that this hormone is probably the same as that emanating from leaves to promote normal cambial activity. In terms of this suggestion, it is implied that the hormone moves to some extent in advance of the normal basipetal development of cambial activity, which is indeed not at all unlikely. That the hormones promoting normal cambial activity and wound cambial activity are probably one and the same is also supported by the response of these two processes to the influence of gravity. In both cases, development is greater on the upper than on the lower side. Finally, cambial activity in relation to wounding, apart from that obtaining at the

lower edge of a complete ring, is not so very different from normal cambial activity. The same types of elements are produced in both cases, at all events so far as the xylem is concerned, and presumably in the phloem also. If a hormone is involved in normal cambial activity, it would therefore appear reasonable to suspect hormone action in relation to wound cambial activity, and any evidence in that direction assumes significance.

In order to explain the response of both normal and wound cambial activity to gravity, it would appear to be necessary to postulate accumulation of the hormone on the upper side of horizontally placed shoots. There arises in this connection, quite independently of wound cambial activity, a very interesting problem which has not hitherto been stressed. Snow (23) has indicated that cambial activity and cell extension in stems are probably promoted, under natural conditions, by the same growth hormone, viz., auxin-a. According to the well known Cholodny-Went theory of geotropism, the growth hormone accumulates on the lower side of plants placed in the horizontal position, and this has indeed been shown to be true experimentally. On the other hand the cambial hormone, which according to Snow is in all probability identical with the growth hormone, must apparently accumulate on the upper side of leader shoots of woody dicotyledons when they are placed horizontally. However the new extension growth of horizontally placed leader shoots of woody dicotyledons is definitely negatively geotropic, so that if the cambial hormone and the growth hormone promoting cell extension and geotropic curvature are identical, we are forced to conceive of the hormone accumulating on the lower side in the region of negatively geotropic curvature, in terms of the Cholodny-Went hypothesis, and then on the upper side in older portions of the shoot in order to explain the observed relation between cambial activity and gravity. This is but one of many paradoxes to be found in the field of plant hormones, which are, however, well worth stressing, if only in order to offset any tendency towards acceptance of an as yet unwarranted simple relation between plant hormones and the processes with which they are connected.

Sledge (20) has suggested that a wound stimulus, functioning through the injection with sap of the intercellular spaces in the vicinity of the cambium, plays an important part in the initiation of basifugal cambial activity at the base of woody cuttings. He then goes on to say that the absence of cambial activity at the distal end of cuttings, where presumably the same conditions of sap injection must hold, and which cannot always be accounted for by a basipetal transport of food, points to the existence of another "unknown factor" which is also involved. It has been shown in the present investigation that a very limited amount of basipetal cambial activity does arise from the lower edge of a complete ring in balsam poplar. However, it would still appear to be necessary to postulate Sledge's "unknown factor" in order to explain, not only the great difference in the amount of cambial activity just above and below a complete ring in completely disbudded units, but in addition, the marked contrast in differentiation subsequent to cell division

in the two cases. In terms of the suggested explanation of the quantitative results obtained in this present investigation, the "unknown factor" is now interpreted as being a hormone.

The question of food supply as a possible alternative to the hormone hypothesis calls for a certain amount of discussion. Sledge (20) came to the conclusion that polarization of wound cambial activity in woody cuttings could not be accounted for as the result of a basipetal movement of food material. The same opinion with regard to normal cambial activity is shared by Snow (21, 22, 23) and others. Loomis (12) found that the relation between active leaves and cambial activity in the shoot below could not be correlated with the production of carbohydrates by the leaves, since carbohydrate levels were higher in sprouting segments which showed but little cambial activity, than in leafy segments where cambial activity was marked. Then again, Priestley and Tong (16) found that, although there was definitely more starch on the upper side of horizontal branches of Tilia and Acer showing marked epitrophy (i.e., greater radial growth on the upper relative to the lower side), in Crataegus which also shows marked epitrophy of its horizontal branches, starch was deposited more heavily on the lower side. In the present investigation no special effort was made to follow the movement and distribution of starch, but it was observed that there was definitely less starch in units bearing developing buds and leaves than in contrasting units which had been disbudded, whereas wound cambial activity was more marked in the former. It is of interest in this connection to note that Loomis (12) has shown that during the earlier development of leaves and sprouts from buds, the movement of food materials tends to be upwards. On the whole, therefore, it would appear that a more satisfactory explanation of the quantitative results obtained in this work is to be found in terms of hormone action, rather than food supply.

The marked swelling of preformed root initials on the upper side only of horizontally placed units of balsam poplar is of more than passing interest. It has been shown by Thimann and Koepfli (26) and also by Cooper (5), that root formation is promoted by the same growth hormones that bring about cell extension in stems, and Laibach (11) reports that hetero-auxin promotes both the development of roots and callus tissue in stems. In other words the same growth hormones promote cell extension, root formation and cambial activity, as well as a number of other processes which need not be detailed here. We have therefore the interesting correlation between stimulation of root initials and the greater development of wound cambial activity on the upper side of horizontally placed shoots, which does in a measure support the writer's suggestion that the hormone promoting normal cambial activity is also involved in the development of local wound cambial activity.

#### (b) Qualitative Results

It will probably be admitted that the observed behavior of the cambium in relation to wounding cannot be explained satisfactorily, simply in terms of the accumulation or concentration of a hormone moving basipetally down the shoot. Consider for example the longitudinally bridged wound (Figs. 2 and 3). In this case the hormone will tend to accumulate above the upper transverse margin, but the experiment illustrated in Plate I, Fig. 1 indicates that lateral translocation takes place quite readily. One would therefore expect to find a greater concentration of hormone within the bridge than just below it. Nevertheless it has been shown definitely that there is relatively more cambial activity in the obliquely basipetal development below the wound, than occurs within the bridge itself. In other words, the amount of cambial activity is not wholly determined by the concentration of hormone. Then again, consider the absence of cambial activity in the region A (Figs. 2 and 8), and the occurrence of cambial activity lateral to it, where presumably the concentration of hormone is lower. Apparently therefore, some other factor plays a part, something no doubt of the nature of a wound stimulus.

At this point the author would like to stress particularly the fact that extreme care was taken in the making of wounds, in relation to which critical observations of the type to be discussed in this section were obtained. The importance pertaining to discontinuity of the elements of the bark as a result of transverse incisions was early recognized, and special care was taken to avoid cutting into the bridge itself, particularly at the basal end. Moreover a common practice employed, which is indicated in many of the figures, was to make longitudinal bridges and the like slightly wider at the basal end than at the distal end. In a word, the writer is satisfied that the observations to be discussed now are truly significant and not the result of imperfect technique, which as a matter of fact is entirely precluded in certain developmental relations showing definite homologies with other cases into which the question of technique might conceivably enter.

The conception of a wound stimulus in relation to wound cambial activity is not without precedent. Sledge (20) postulates a wound stimulus in his work, and conceives of it as functioning through the injection with sap of the intercellular spaces in the vicinity of the wound. Such an explanation is not, however, in the opinion of the present writer, sufficient to explain the observed behavior. It would imply less sap injection within the bridge than below it, which might be possible, but it would not explain the absence of cambial activity at the point A (Fig. 2), where presumably the same conditions of sap injection must exist as in the region lateral to it where cambial activity does occur. Similarly it could not explain the behavior depicted in Fig. 8.

Only one attempt has ever been made hitherto to explain development below bridged wounds and the like, and it is to be found in the work of Janse (10). This investigator was interested primarily in the following question. Does there exist in the plant a force or action which has the tendency to drive or push substances always and invariably in a given direction, ordinarily downwards? As the result of a series of wounding experiments, he came to the conclusion that there was such a force and he called it the "force spéciale," a term which he ultimately replaces by "impulsion basipétale." He observed

the obliquely basipetal development of cambial activity below bridged wounds, and he interpreted this phenomenon as the result of the action of two other forces, viz., the "attraction du cambium" and the "attraction des tissus traumatiques," upon his third force, the "force spéciale" or "impulsion basipétale." Let us now consider a particular case, for example, development below a rectangular wound of the type depicted in Fig. 4. Janse's interpretation would be as follows. Below the lower transverse margin of the wound two forces are to be considered, (i) the "attraction du cambium" which acts in a horizontal direction simply because the margin of the wound is transverse (if the wound margin were oblique the direction of this force would also be oblique, according to Janse), and (ii) the "attraction des tissus traumatiques" which acts perpendicularly to the wound margin. These two forces set up, in the region below the wound, a so-called field of attraction which reacts upon the "impulsion basipétale" acting longitudinally from above downwards. in such a way as to cause lateral diversion of the "impulsion basipétale" below the wound. As a result of this, food material is also diverted laterally in this region to nourish the cambium in its path. He does however write as follows: "Il faut se rappeler cependant ici que l'afflux de nourriture ne doit être considéré comme la cause du développement des tissus, mais que c'est au contraire la division des cellules et leur agrandissement qui sont les causes du courant qui va se diriger vers elles." But nevertheless throughout his paper he clearly considers food supply to be the important factor in development, subsequent to the change in direction of the "impulsion basipétale."

Janse's interpretation is decidedly obscure, if only by dint of lack of definition of his forces. His "impulsion basipétale" may be interpreted as being simply the recognized tendency for cambial activity to develop normally in the basipetal direction, and it is generally conceded that the direction may be obliquely basipetal without necessarily postulating the action of contributory forces. He stresses greatly the action of the "impulsion basipétale" upon the downward movement of food materials, whereas the same reaction of the cambium can be observed in relation to wounds in regions where the general movement of food materials is upwards, e.g., in regions bearing developing buds or sprouts (e.f. Loomis (12)). Then again it is by no means clear how Janse would explain the absence of cambial activity in the region A (Figs. 2 and 8) in terms of the interaction of his three forces.

The present writer is also of the opinion that a wound stimulus is involved in cambial activity in relation to wounding, and ventures to suggest that a satisfactory explanation is to be found in the conception of this wound stimulus in terms of a definite wound substance. This hypothesis is based upon the observations made in this investigation and upon certain very significant correlations. It will first of all be stated briefly. The main points are as follows:

 Subsequent to wounding, a definite wound substance is produced as a result of death or dying of the cells bordering upon the wound.

- (2) The amount of this wound substance produced is proportional to the extent of dying.
- (3) The wound substance can of itself promote cell division only, in the cambial layers.
- (4) Differentiation of more or less typical xylem and phloem, and in addition further cell division, is promoted by the cambial hormone which moves basipetally down the living bark and reacts with the wound substance to give the observed behavior.

In the discussion to follow, the writer proposes at times to discuss his observations in terms of the hypothesis, and at other times the hypothesis will be discussed in terms of the observations and correlations which led to its formulation. In terms of the hypothesis the marked contrast in local cambial activity at the upper and lower margins of a complete ring is readily understandable. Development at the lower margin is to be attributed almost solely to the action of the wound substance, causing cell division only in the cambial layers. The absence of differentiation (with the exception of a few tracheids) to form vessels and fibres is the result of depletion in this region of the cambial hormone, on account of its basipetal transport down the shoot. Cambial activity involving differentiation of xylem just above a complete ring is the result of interaction between the wound substance and the cambial hormone which moves basipetally towards the wound margin from above. However the difference between cambial activity at the upper and lower margins of a complete ring is not simply the presence of vessels and fibres in the one case and their absence in the other. It is clear that the cambium divides more frequently above a complete ring than below it, so that one has to conceive of the cambial hormone as promoting not only differentiation of xylem elements, but also, as might indeed be expected, further cell division.

Consider now behavior in relation to the type of wound depicted in Fig. 1, in which a longitudinal tongue of bark runs distally from the lower margin of a complete ring. Here again there is little or no differentiation of vessels and fibres, on account of basipetal transport of the cambial hormone. The interesting points are that stimulation of cambial activity is greater longitudinally from the transverse margins than laterally from the longitudinal margins, and the exceedingly suggestive correlation that dying of the cells subsequent to wounding is also more extensive longitudinally from the transverse margins than laterally from the longitudinal margins. Hence the hypothesis to the effect that production of wound substance is proportional to dying subsequent to wounding.

It has been shown that there is definitely more cambial activity above and below a longitudinally bridged ring than occurs within the bridge itself (Figs. 2 and 3). The correlation that immediately suggests itself here is that dying subsequent to wounding is more extensive longitudinally from the upper and lower transverse margins of the wound than laterally from the longitudinal margins, which implies that there will be more wound substance produced

just above and below the wound than within the longitudinal bridge. On this basis therefore, it is suggested that the observed behavior might be interpreted as the result of a low concentration of wound substance within the bridge acting as a limiting factor, with the proviso that the limiting action is relative, not absolute. Precisely the same interpretation can be applied to behavior in relation to Czapek's ring (Figs. 6 and 7), where again there is relatively more cambial activity in the obliquely basipetal development below the wound than occurs within the vertical strips of the zig-zag bridge. On the other hand the condition just discussed is not apparent in relation to the obliquely bridged ring, which is just what might be expected, since dying longitudinally from a definitely oblique wound margin is of the same order as that obtaining from a transverse margin. One would not therefore expect to find wound substance limiting within the oblique bridge.

It still remains to explain a peculiar feature of the obliquely basipetal development below bridged wounds and the like. Let us consider specifically the longitudinally bridged wound (Fig. 2), although behavior below all bridged wounds is essentially similar. Below the lower transverse margin of the wound there is depletion of the cambial hormone as a result of its basipetal transport, similar to that obtaining below a complete ring. However there is still a supply of this hormone arriving from above by way of the longitudinal bridge, below which it spreads out obliquely basipetally on both sides from the region of relatively high concentration in the same longitudinal line as the bridge into the depleted region below the lower transverse margin of the wound. The question then arises, why is there no cambial activity in the region A (Fig. 2), whereas cambial activity occurs lateral to it, where presumably, the concentration of cambial hormone must be lower. In order to explain this, the writer suggests that the wound substance emanating from the lower transverse wound margin is used up when it reaches and interacts with the cambial hormone, at what might for convenience be termed the "diffusion" front of the cambial hormone, in terms of which, the absence of cambial activity in region A is again to be interpreted as the result of lack or low concentration of wound substance acting as a limiting factor. At points lateral to A, where cambial activity does occur but which are equally far removed from the lower transverse margin of the wound as A, wound substance has not been limiting, simply because it has been free to move down that far before meeting and interacting with the cambial hormone at its obliquely basipetal "diffusion" front.

The exceedingly interesting behavior observed in the modified zig-zag bridge (Fig. 8) can also be readily interpreted along the same lines, in the following manner. The obliquely basipetal development of cambial activity across the lower vertical strip is the result of interaction between the wound substance emanating from the transverse margin just about it and the cambial hormone at its obliquely basipetal "diffusion" front, in exactly the same manner that obtains below the bridge in the same specimen. The basipetal development down the shorter longitudinal margin of the lower vertical strip is similar

to that within a longitudinal bridge, and the absence of cambial activity in the region A is again due to a low concentration of wound substance acting as a limiting factor. There would seem to be little doubt in this case but that the concentration of cambial hormone must be higher in region A than at points lateral to it on the left, where cambial activity does occur. is suggested that the wound substance emanating from the transverse margin of the lower vertical strip is used up in interaction with the cambial hormone just above and lateral to the left of region A, and there would seem to be little possibility of wound substance reaching this region from the longitudinal margin to the right, since what little of it is produced there (c.f. longitudinally bridged wound) is used up in the basinetal development of cambial activity down that margin. To summarize then, local cambial activity in relation to bridged wounds and modifications of such is to be interpreted as the result of interaction between a wound substance, produced subsequent to wounding, and the cambial hormone which is present in the living bark and moves basipetally down the shoot. At certain points in relation to a wound, a low concentration of wound substance may act as a limiting factor, but its limiting effect is only relative, since cambial activity is further promoted at all points by increased supplies of the cambial hormone (c.f. quantitative experiments).

Although interaction between a wound substance and a hormone is, so far as the writer is aware, a new interpretation of local cambial activity in relation to wounds, the idea is not without precedent with regard to certain other growth processes. Haberlandt (6, 8) found that cell division at the cut surface of potato tubers was most marked in the immediate vicinity of vascular strands. The xylem of the bundle did not seem to play any part, all that was necessary was phloem tissue. Pieces of potato which did not contain any vascular tissue also showed cell division, but not to the same extent as when phloem was included. If a piece without bundles was separated from another piece with bundles by means of a thin film of agar, then cell division at the surface of the former was increased. He came to the conclusion that cell division in pieces of tissue containing vascular bundles was the result of interaction between a hormone produced in the phloem and a wound hormone produced subsequent to wounding. A summary of Haberlandt's work along these lines is also to be found in a paper by Pringsheim (17). Then again, Nakano (13) came to the conclusion that besides correlative influences, the interaction of two hormones was also involved in the production of typical callus tissue. On the other hand, it is of interest to find that Cholodny (3) has attempted to explain certain experimental results by postulating that a wound hormone retarded growth, by neutralization of the effect of the growth hormone.

Haberlandt (7, 8) found that he was able, not in every case however, to limit the amount of cell division subsequent to wounding, by washing the wound surface thoroughly in a stream of water. The present writer performed similar experiments, which need not be described in detail here, with wounded

balsam poplar. The wound surface was washed for 10-15 minutes in a stream of tap water, and then for a few minutes in distilled water. No evidence was obtained that the amount of cambial activity in relation to the wound was in any way affected as a result of washing, and the extent of dying subsequent to wounding was, as far as could be determined, of the same order in washed and unwashed wounds.

In the present paper the writer has refrained from designating the wound substance as a wound hormone, since apparently there is some doubt as to the legitimacy of so doing. Petri (14), in a recent paper, concluded that the mode of origin and properties of so-called wound hormones seemed to preclude of their being so defined, even according to the concept of plant hormones. He has suggested that they be considered as an oxidation product of a compound normally present in living cells.

Before concluding, a few remarks relating to further research on the subject of local cambial activity as a result of wounding might be in order. At the present time much is becoming known about the isolation and purification of plant hormones, and in some cases synthesis has been effected. Snow (23) has succeeded in promoting cambial activity by the use of pure hormones. The response was obtained just below the point of application of the hormone. With regard to wound cambial activity on the other hand, the ideal experiment would be one in which a response is obtained in the immediate vicinity of the wound, as a result of application of the hormone at a point some distance from the wound, and in which the response at the wound is more or less distinct and separate from any development arising at the point of application. Stimulation of root development in regions considerably removed from the point of application of the growth hormone has been obtained by a number of investigators, some of whom have already been mentioned (c.f. Laibach, Thimann and Koepfli, and Cooper), but nothing is known about the response of the cambium in these experiments. Should, however, an experiment of the type just outlined be successfully performed, the whole problem of certain other relations, discussed in this paper in terms of interaction between the cambial hormone and a wound substance, would still remain, so that further experimentation is called for in this connection also, in an attempt to determine more definitely the nature of the wound stimulus. The writer himself hopes to be able to continue his experiments along the lines indicated, and it may be that the results obtained in this investigation, and the interpretations suggested are of sufficient interest to stimulate research by other workers.

# Acknowledgment

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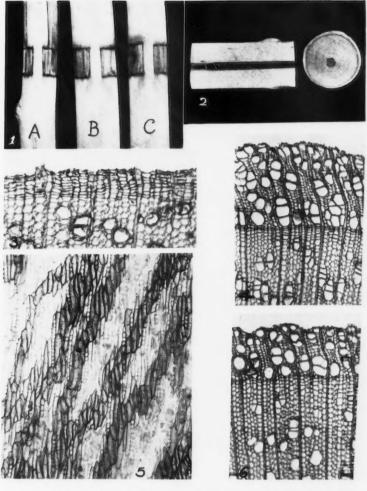


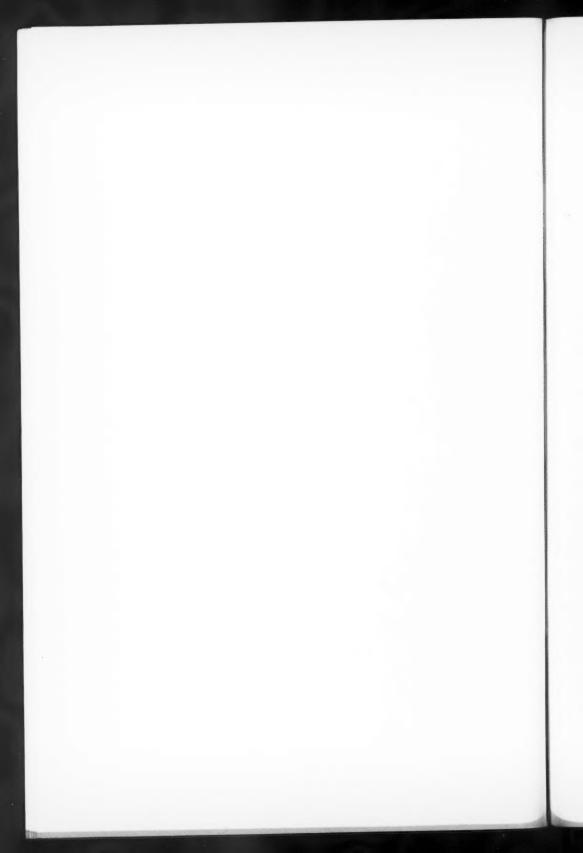
Fig. 1. Three units of balsam poplar in 3-year-old wood obtained from the same leader shoot, illustrating the relation, in terms of xylem formation, between the extent of cambial activity in the vicinity of a wound and the amount of living bark distal to the wound. Four-fifths natural size.

Fig. 2. Illustrating in longitudinal and transverse section, the effect of gravity upon local cambial activity just distal to a complete ring, as revealed when the bark is peeled from the shoot. Cambial activity is more marked on the upper side. X 1.6.

Fig. 3. Illustrating wound cambial activity just below a complete ring. Transverse section of a peeled shoot just below a complete ring, showing several layers of more or less regularly rectangular cells cut off to the inside by the vascular cambium. X 128.

Figs. 4 and 6. Xylem formation at corresponding points within the bridge of a longitudinally bridged ring in two contrasting units. In one unit (Fig. 4) two buds at the distalend were allowed to develop, whereas the other unit (Fig. 6) was completely disbudded. About one-third of the bridge, cut transversely, is shown in each photograph. X 96.

Fig. 5. A sheet of xylem from the obliquely basipetal development below a bridged wound, X 88.



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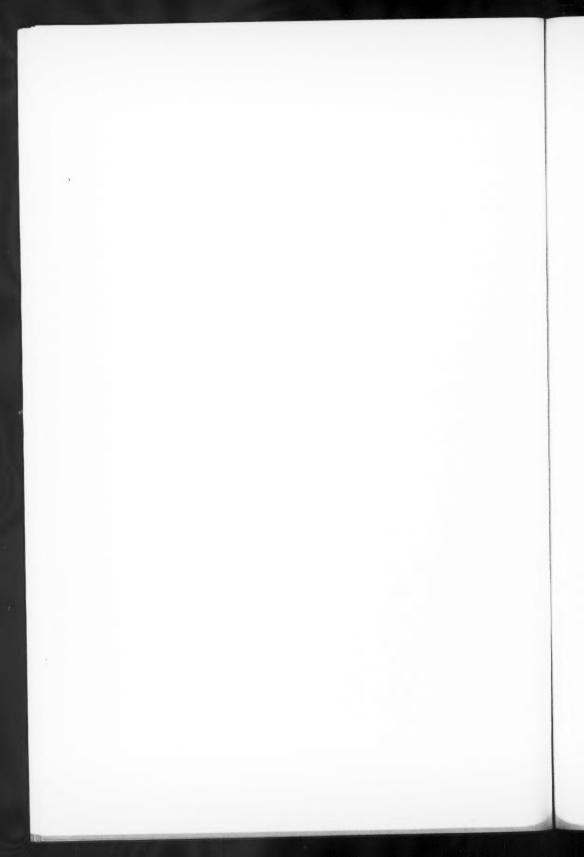
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## THE INTERRELATION OF BLOOD LIPIDS1

By Eldon M. Boyd2

## Abstract

The relation of the concentration of neutral fat, phospholipid, free cholesterol and cholesterol esters of blood plasma, the red blood cells and the white blood cells of man to increasing percentages of total lipid in each of these respectively was determined by oxidative micromethods in a series of more than 900 lipid extracts. A parallel increase in all four lipids was noted with increasing amount of total lipid in plasma until the total lipid exceeded the normal range. The earliest manifestation of a lipemia was a precipitous increase in plasma neutral fat and no increase or a decline in plasma cholesterol esters which later rose again as the lipemia developed. Increasing amounts of total lipid in the red blood cells was found due chiefly to phospholipid; although when the total lipid exceeded the normal range appreciable amounts of neutral fat appeared, the increase in cellular lipids bearing no direct relation to increases in plasma lipids. The same relation was established for the white blood cells as for the red blood cells but the leucocytes contained considerably more lipids than either plasma or the red blood cells.

A correlation has been found between the concentration of the several lipids present in blood in a number of situations. It is beyond the scope of this paper to discuss previous results where only two or three such lipids have been compared. The following is a brief review of previous typical studies in which all or nearly all of the known blood lipids have been considered. In 1921, Bloor (2) described the lipaemias of diabetes mellitus and of persistent hemorrhage as associated with increases in all of the plasma lipids. Similar approximately parallel changes in lipid percentages of plasma or serum were found by Bloor (3) and by Bender and Maynard (1) in relation to changes in diet; by Maynard, Harrison and McCay (26) and by Schaible (31) in relation to lactation; by Man and Peters (25) in relation to diabetic acidosis; by Stoesser and McQuarrie (34) and by Boyd (10) in relation to fever; by Page, Kirk and Van Slyke (30) and by Page and Farr (28) in relation to renal disease; by Boyd (9, 14) and by Boyd and Fellows (19) in relation to pregnancy; and by Boyd (11) in relation to the puerperium. In whole blood a parallelism was noted by Chaikoff, McGavack and Kaplan (23), but Okey and Boyden (27) failed to find one in their studies. Few attempts have been made to correlate changes in the red blood cells; Boyd (10) found a tendency towards parallel changes in fever. The author has examined the lipid content

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of the white blood cells in a number of conditions (6–8, 13, 16, 17, 20, 22) but has not hitherto recorded any possible relation of leucocytic lipid values to their total lipid.

The method used in the present work consisted in tabulating according to their total lipid values a large number of complete, differential lipid analyses of plasma, the red blood cells and the white blood cells of human blood. These samples of blood were from persons afflicted with a wide variety of diseases and from subjects who were found to have no condition known to affect the lipid values of blood. The numerous analyses were divided into groups of increasing total lipid values, the mean values of total lipid and component lipids were determined in each group, along with the standard deviations of the means, and an attempt was made to correlate the concentrations of the component lipids with that of the total lipid. In this manner it was possible to ascertain the average part played by each of the component lipids in the development of a lipaemia or of a lipopenia in plasma and to obtain some conception of the relative function of the lipids of the red cells and of the white cells in relation to their total lipid. Wide variations found in the values of certain of the cellular lipids rendered invalid definite conclusions based on comparisons of means. It may be noted that the term, lipopenia, was introduced by Boyd (10) to describe the occurrence of a general decrease in blood lipid values.

Oxalated or citrated blood was used and was taken usually from fasting subjects although it was not possible to insist upon fasting conditions in all cases. All of the subjects were on a standard, hospital diet, and under these conditions meals have been shown by Boyd (12) to have little influence upon the concentration of plasma lipids of normal persons although they may influence to some slight extent the lipid content of the red blood cells (21). It has been shown that oxalated, and presumably also citrated, plasma contains roughly 10% less lipid than heparinized or defibrinated plasma (18) while oxalated red blood cells contain proportionately more lipid than do red blood cells from defibrinated blood (15). Extracts were prepared and analyzed by Bloor oxidative micromethods as modified by Boyd (4, 5).

#### The Lipids of Plasma

The interrelation of blood lipid values will be considered under three main headings, the lipids of plasma, of the red blood cells and of the white blood cells. The interrelation in human blood plasma was determined from 421 complete, differential analyses representing over 4,000 lipid values. The series included 118 differential analyses on normal subjects, the remainder being of abnormal conditions. The lowest plasma total lipid was 100 mg. per cent (milligrams of total lipid per 100 cc. of plasma) and the highest was 1532 mg. per cent. The series was divided into 12 groups, Group 1 including cases with a plasma total lipid up to and including 199 mg. per cent, Group 2 from 200 to 299 mg. per cent, Group 3 from 300 to 399 mg. per cent, etc.

## The Interrelation of All Cases

An analysis of these lipid values (approximately 4,000) has been presented statistically in Table I. In this and in succeeding tables and figures, the value of cholesterol ester has been calculated as the sum of ester cholesterol plus

TABLE I

The lipid composition of 421 samples of human blood plasma grouped according to the value for total lipid of each complete, differential analysis (The lipid values are expressed in mg. per 100 cc. of blood plasma)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos- pholipid	Free cholesterol	Cholestero ester
		Minimum	100	13	21	5	13
		Maximum	185	91	90	29	52
100-199	17	Mean Standard deviation	138	42 20	52 21	13	31 13
		Standard deviation in per cent of mean	20%	48%	40%	54%	42%
				70	/6		70
		Minimum	200	0	28	11	18
		Maximum	298	201	139	39	114
200-299	17	Mean	251	94	74	22	51
		Standard deviation Standard deviation in per	32	61	36	9	28
		cent of mean	13%	65%	48%	41%	55%
		Minimum	303	0	42	6	2
		Maximum	398	283	191	42	185
300-399	51	Mean	359	102	116	3.3	108
		Standard deviation Standard deviation in per	. 29	47	27	8	35
		cent of mean	8%	46%	23%	24%	33%
		Minimum	404	28	52	28	0
		Maximum	499	270	200	90	245
400-499	82	Mean Standard deviation	458	129 51	140 28	43	146 41
		Standard deviation Standard deviation in per cent of mean	28	40%	28	23%	28%
		Minimum	502	0	105	23	97
F00 F00	02	Maximum Mean	599	300 145	235	65	299
500-599	92	Standard deviation	549 32	49	170 30	48	186 39
		Standard deviation in per					
		cent of mean	6%	34%	18%	19%	21%
		Minimum	602	11	113	27	65
		Maximum	695	437	259	93	419
600-699	60	Mean	648	186	198	55	209
		Standard deviation Standard deviation in per	28	70	27	13	56
		cent of mean	4%	38%	14%	24%	27%
		Minimum	703	90	156	45	66
Mac 800		Maximum	798.	408	285	100	272
700-799	27	Mean	746	254	219	66	207
		Standard deviation Standard deviation in per cent of mean	4%	71 28%	32 15%	21%	53 26%

TABLE I-Concluded

The lipid composition of 421 samples of human blood plasma grouped according to the value for total lipid of each complete, differential analysis (The lipid values are expressed in mg. per 100 cc. of blood plasma)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos- pholipid	Free cholesterol	Cholestero ester
		Minimum	801	97	151	47	0
		Maximum	896	528	422	136	367
800-899	33	Mean	847	325	248	76	198
		Standard deviation Standard deviation in per	30	80	46	21	80
		cent of mean	4%	25%	19%	27%	40%
		Minimum	901	242	173	60	99
		Maximum	997	570	314	92	389
900-999	17	Mean	947	341	261	76	269
		Standard deviation Standard deviation in per	30	82	39	8	63
		cent of mean	3%	24%	15%	11%	23%
		Minimum	1012	168	192	40	217
		Maximum	1082	518	352	108	569
1,000-1,099	10	Mean	1053	384	292	81	296
		Standard deviation Standard deviation in per	24	103	42	18	97
		cent of mean	2%	27%	14%	22%	33%
		Minimum	1103	230	268	95	130
		Maximum	1231	622	336	100	499
1100-1299	6	Mean	1142	441	313	98	290
		Standard deviation Standard deviation in per	42	103	33	2	168
		cent of mean	4%	23%	11%	2%	58%
		Minimum	1329	382	255	90	269
		Maximum	1532	773	510	123	481
1300 and over	9	Mean	1401	540	393	107	361
		Standard deviation Standard deviation in per	65	116	75	10	68
-		cent of mean	5%	21%	19%	9%	18%

cholesterol ester fatty acid as appeared in previous presentations of results (e.g., 5). The method of determining and calculating the several lipids listed in these figures and tables has been previously described (5). In offering the results in this manner, the author has had in mind the simplification of description of data on blood lipids. As blood lipids are understood at present, there are four main groups; namely, the neutral fats, phospholipids, cholesterol esters and free cholesterol. Such values as ester cholesterol, total cholesterol, total fatty acids, etc., are convenient laboratory expressions since they represent values that may be determined by methods of analysis. When only part of a complete lipid analysis is performed, these laboratory values must needs be reported. When different methods of analysis are compared, it is also preferable to compare experimentally determined rather than calculated values. But for purposes of description such as is aimed at herein, these laboratory expressions may well be omitted. Such additional data may be calculated if desired, from the information given in the several tables, by applying the factors previously discussed (5).

The statistical analysis of results presented in Table I includes the listing of the minimal value for each lipid in each of the groups, the corresponding maximal value, mean value, standard deviation of the mean and, for purposes of comparing relative variations, the percentage standard deviation or the standard deviation expressed as a percentage of the mean. The standard deviation was calculated from the formula:

$$\varphi = \sqrt{\Sigma(x)^2/n}$$

where  $\varphi$  represents the standard deviation,  $\Sigma$  a summation symbol, x the difference between each individual value and the mean value and n the total number of values considered in the mean. To obtain the value of the standard deviation, each individual value was subtracted from the mean, these differences were squared, added together, divided by the total number of values and the square root of the resulting figure gave the standard deviation. From the mean and the standard deviation the expected range of values may be calculated. Thus two-thirds of values may be expected to lie within the range of the mean plus and the mean minus the standard deviation while 95% of all expected cases lie within the range of the mean plus and minus twice the standard deviation.

There was comparatively little variation in the values for *total lipid* in each of the groups given in Table I. The greatest relative variation occurred in the first group of 100–199 mg. per cent total lipid. In this group the mean was 138 mg. per cent and the standard deviation 27, which was 20% of the mean. The percentage standard deviation declined to 13% in the second group, to 8% in the third group, to 6% in the fourth group, and from then on it remained at low values between 2 and 5%. The percentage standard deviation for total lipid was lower in all except the first two groups than the percentage standard deviation of practically all the other lipids. The reason for this was that total lipid values were selected within a certain range and hence were not subject to the same chance variation as the other lipids. The standard deviations for the means were approximately the same in most of the total lipid groups, and since the means for total lipid became progressively greater, the percentage standard deviation obviously had to become progressively less.

Values for neutral fat were found to be amongst the most variable of all plasma lipids. The standard deviation may be seen from Table I to extend from 21 to 65% of the respective means. The neutral fat of human plasma was found to range from none at all to as high as 773 mg, per cent. In one group the range of values for neutral fat was from 0 to 201 mg, per cent although the total lipid figures of this group covered a range of only 98 mg, per cent. Neutral fat may enter plasma either from the tissues which store fat (neutral fat is the chief type of storage fat) or from the intestine during fat absorption. Part of it at least is carried to the liver where it is probably converted into phospholipid and cholesterol esters. During extensive parenchymatous damage to the liver, the author has noted a marked decrease in the phospholipid and cholesterol ester content of plasma with neutral fat

values as great as, or greater than, normal, suggesting that in this condition the liver failed to convert the neutral fat coming to it into phospholipid and cholesterol esters.

The concentration of plasma *phospholipid* extended from 21 to 510 mg, per cent in the series of cases studied. There was less variation in the phospholipid content in the various groups than occurred in neutral fat. The standard deviations extended from 11 to 48% of the respective means, the greatest variation being again encountered in those groups with lowest mean values. One function of plasma phospholipid has been definitely shown by Sinclair (32) to be the transport of fatty acids. Boyd and Wilson (22) showed that phospholipids transport fat from the placenta to the human foetus in utero.

The free cholesterol content of plasma covered a range of from 5 to 136 mg. per cent. The standard deviation varied between 2 and 54% of the means, being greatest in the groups with lowest mean values. This fraction is usually designated "free" cholesterol to distinguish it from "ester" or "combined" cholesterol and from the sum of these, the "total" cholesterol. It would appear preferable to designate free cholesterol merely "cholesterol" and the other fraction "ester cholesterol" since the nomenclature in common usage has tended to imply that the only function of plasma cholesterol is its role in fat metabolism, and to render obscure the fact that cholesterol may, and probably does, have other important functions such as, for example, the possible relation of cholesterol to the genesis of sex hormones. At present, when merely blood cholesterol is stated, the inference is that blood total cholesterol is meant. Cholesterol is related to lipid metabolism in that it forms esters with fatty acids and it would appear, as previously stated, that this synthesis is effected at least in the liver. Sperry (33) has shown that there is in blood plasma a cholesterol esterase which splits cholesterol esters.

Values for plasma *cholesterol ester* were comparable in their variability to those of neutral fat. The concentration of this lipid extended from 0 to 569 mg. per cent and the standard deviation ranged from 18 to 58% of the respective means. Part at least of the variation in cholesterol ester values is due to the fact that this lipid is calculated from two or more experimentally determined figures, and hence its value would tend to contain a summation of experimental errors. Cholesterol ester is sometimes written in the singular, sometimes in the plural. While there is apparently only one fatty acid group attached to one molecule of cholesterol in the ester, there is little doubt but that in plasma there are many different compounds of cholesterol with various fatty acids.

# The Relation of Component Lipids to Total Lipid of Plasma

In Fig. 1 the mean values of the individual lipids listed in Table I have been plotted against the mean values of the total lipid. The relation of one lipid to another in the lipopenic, normal or lipaemic phases may thus be clearly seen. This figure demonstrates that the three chief lipids of plasma are cholesterol esters, neutral fats and phospholipids and that the fourth

so-called lipid, namely, free cholesterol, is present in much smaller concentrations throughout. In order to give some conception of the relation of these curves to the normal range of values, a "normal range" has been indicated in Fig. 1. This "normal range" is the expected range of 95% of

values for total lipid of normal human adults as taken from Table III. The values of the several curves passing through the normal range in Fig. 1 are not necessarily normal values for these component lipids, since the curves are drawn from mean values of both normal and abnormal values. The relation of normal values for component lipids to their total lipid will be considered later.

Fig. 1 illustrates that the concentration of phospholipid and cholesterol ester follow an almost parallel course from the lowest lipopenic values to about the upper limit of the normal range of total lipid. Beyond this point, phospholipid continued to increase at much the same rate, bearing almost a direct linear relation to total lipid throughout. Cholesterol ester passed through a temporary declining or stationary phase when it reached the upper limit of the normal range, this phase persisting from average total lipid values of from about 600 to 900 mg. per cent. It may be inferred that the onset of a lipaemia is accompanied by a temporary decline in

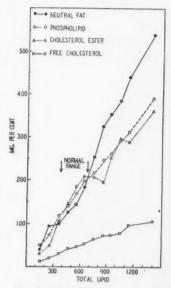


Fig. 1. The relation of mean values of lipids in blood plasma of healthy and diseased persons to mean values of plasma total lipid expressed in mg. per 100 cc.

plasma cholesterol ester values. This peculiar behavior of cholesterol esters is emphasized because it has been recorded in the results of Gardner and Gainsborough (24) and, in somewhat modified form, in the results of Boyd and Fellows (19) in their studies of blood lipids in pregnancy of women and of guinea pigs. Such a phase in cholesterol ester values has not been stressed in previous considerations of the different types of lipaemia wherein it has apparently been missed by insufficiently detailed study. What its significance may be is at present obscure. It indicates that there must be some important difference in the metabolism of this lipid in contrast to the others and refutes the conception (26) that all lipid values of plasma increase at the same rate and at the same time. Following the phase of decline, the cholesterol ester content of plasma increased roughly parallel to that of phospholipid, with the exception of a further decline at total lipid values of about 1000 to 1100 mg. per cent. The second decline may be an artifact or it may indicate a tendency for fluctuation in plasma cholesterol ester values such as are found in hog cholera and in the

lipopenia of fever in man (10), fluctuations which again have not been stressed in most other considerations of plasma cholesterol ester. It is apparent that much detailed study of the changes in the concentration of this lipid in particular and of all lipids in general is necessary before explanations as to their functions should even be attempted.

Values for free cholesterol exhibited, like those of phospholipid, a direct linear relation to total lipid. The similarity in behavior of these two lipids is further evidence in support of the hypothesis that they are closely allied in vital economy, the one being considered the antagonist of the other.

## The Interrelation of Plasma Lipids in Normal Cases

In Table II have been listed values for plasma lipids in 118 samples of blood from normal human adults grouped according to the concentration of total lipid. The lowest figure for total lipid encountered in this series was

TABLE II

The lipid composition of 118 samples of blood plasma from normal human adults grouped according to the value for total lipid of each complete, differential lipid analysis

(The lipid values are expressed in mg. per 100 cc. of plasma)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos- pholipid	Free cholesterol	Cholestero
	1	Minimum	381	28	98	32	102
		Maximum	447	118	186	56	188
To 449	13	Mean	427	79	150	41	157
		Standard deviation Standard deviation in per	20	26	23	10	21
		cent of mean	5%	33%	15%	24%	13%
		Minimum	451	50	97	32	109
		Maximum	499	188	200	62	245
450-499	26	Mean	482	115	156	44	177
		Standard deviation Standard deviation in per	13	35	38	11	51
		cent of mean	3%	30%	24%	25%	29%
		Minimum	502	55	111	23	104
		Maximum	546	223	212	64	219
500-549	36	Mean	522	139	175	44	172
		Standard deviation Standard deviation in per	14	42	27	8	28
		cent of mean	3%	30%	15%	18%	16%
1		Minimum	551	85	105	35	132
		Maximum	593	213	210	63	284
550-599	16	Mean	573	164	164	48	197
		Standard deviation Standard deviation in per	17	38	31	8	38
		cent of mean	3%	23%	19%	32 62 44 11 25% 23 64 44 8 18% 35 63 48 8 17% 27 88 51 18 35% 38 74	19%
1		Minimum	602	125	113	27	65
		Maximum	645	437	228	88	254
600-649	18	Mean	623	185	181	51	206
		Standard deviation Standard deviation in per	15	74	25	18	47
		cent of mean	2%	40%	14%	35%	23%
1		Minimum	650	33	124	38	172
		Maximum	752	317	236	74	419
Over 649	9	Mean	683	202	187	53	241
		Standard deviation Standard deviation in per	34	81	33	13	70
		cent of mean	5%	40%	18%	25%	29%

381 mg. per cent and the highest 752 mg. per cent. The variation of total lipid values, as seen from the percentage standard deviation, was lower than that shown in Table I, which was to be expected since the results given in Table II were from a more homogeneous group of subjects. Plasma neutral fat extended from 28 to 437 mg. per cent and its standard deviation from 23 to 40% of the respective means, this lipid being the most variable in value of all lipids in the plasma of normal human adults. Phospholipid was the least variable of the component plasma lipids, the standard deviation ranging from 14 to 24% of the means. The free cholesterol values were almost as constant as those of phospholipid, the standard deviation extending from 17 to 35% of the respective means. Cholesterol esters extended from 102 to 415 mg. per cent and the standard deviations varied between 13 and 29% of the means.

For purposes of comparison with the preceding curves for both normal and abnormal lipid values in Fig. 1, a similar plotting of mean values against total lipid of normal subjects only has been offered in Fig. 2. It may be seen that the component lipid values of normal human plasma bore a relation to total lipid similar to that of all lipid values within the normal range of total lipid indicated in Fig. 1. The most notable differences were, first, a tendency for neutral fat to increase at a rate greater than that of the other lipids over the whole normal range of total lipid; and second, the concentration of cholesterol esters, although exhibiting fluctuations, did not enter the phase of decline

as early as that seen in the plotting of both normal and abnormal cases in Fig. 1. These comparisons would suggest that with further study it may be possible to predict the imminent possibility of a lipaemia developing by a lipid analysis of plasma which may show a normal total lipid, a relatively high neutral fat and a normal or even low cholesterol ester, suggesting that a lipaemia is impending. The close parallelism between phospholipid and free cholesterol is even more evident in Fig. 2 than in Fig. 1. The data presented in Fig. 2 resemble a similar presentation of normal values by Page, Kirk, Lewis, Thompson and Van Slyke (29) in their Fig. 9. Their values for neutral fat are higher than those shown in Fig. 2 and Table II because their method of analysis gave higher values than the oxidative method.

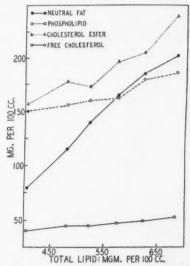


Fig. 2. The relation of mean values of lipids in blood plasma of normal healthy human adults to mean values of total lipid expressed in mg. per 100 cc.

The Normal Range of Plasma Lipids

The analyses of the plasma of healthy human adults have been considered as a whole in Table III in order to establish the normal range of lipids by oxidative micromethods based upon a large number of observations. These subjects were of both sexes, and a tabulation of results according to sex

TABLE III

THE LIPID COMPOSITION OF BLOOD PLASMA FROM 118 DIFFERENTIAL ANALYSES ON NORMAL HUMAN ADULTS

(The results are expressed in mg. per 100 cc. of plasma)

Value	Total lipid	Neutral fat	Phos- pholipid	Free cholesterol	Cholesterol ester
Minimum values*	381	28	97	23	65
Maximum values*	752	437	236	88	419
Mean values	530	142	165	46	177
Standard deviation	74	60	28	8	41
Standard deviation in per cent of mean, %	14	42	17	17	23
Expected range of 2/3 of normal subjects	456-604	82-202	137-193	38-54	136-218
Expected range of 95% of normal subjects	382-678	22-262	109-221	30-62	95-259

<sup>\*</sup>The sum of the individual lipids does not equal the total lipid because the individual lipid values are taken from different analyses.

indicated that there were no significant differences between the lipid values of plasma of men and of women, providing that blood was not taken during menstruation, the climacteric, or pregnancy in women. The age of the subjects listed extended from 15 to 78 and within this range increasing age did not affect the concentration of plasma lipids. Below the age of 15 and especially in infancy and the newborn there was found a lipopenia but such results have not been included in the tabulation of normal values for healthy human adults. The author has previously reported normal plasma lipid values in occasional small groups of cases (5, 9, 12). These are included with many additional values in Table III. This larger group of normal cases revealed means similar to those previously found, except that the values were somewhat lower, especially for phospholipid.

The minimal and maximal values found for plasma *total lipid* were 381 and 752 mg. per cent. The extremes, which may be calculated to include 95% of all expected values, were 382 and 678 mg. per cent. The mean total lipid was 530 mg. per cent and the standard deviation of the mean was 74. In other words human plasma contains roughly from 0.4 to 0.7% of fatty substances or about the same as the amount of inorganic salts present and much more than the water soluble organic compounds such as the nitrogenous derivatives and glucose. In relation to amount present, the lipids of plasma are exceeded only by the albumins and globulins and are about equal in concentration to that of fibrinogen. The relation of this relatively large con-

centration of lipids to colloidal osmotic pressure of plasma and to the genesis of oedema has not received the study it should warrant, owing to its being in colloidal solution.

The total lipid of plasma was composed, on the average, of 33% of cholesterol esters, 31% of phospholipids, 27% of neutral fats and 9% of free cholesterol. The lipid most variable in amount may be seen from Table III to be neutral fat which yielded an expected range for 95% of expected values between 22 and 262 mg. per cent, a mean of 142 mg. per cent and a standard deviation of 60. The variability of cholesterol esters was smaller than that of neutral fat but greater than that of phospholipid and free cholesterol. The minimal value found for cholesterol esters was 65 and the maximal 419 mg. per cent, the mean 177 mg. per cent, the standard deviation 41 and the expected range of 95% of cases was calculated to be from 95 to 259 mg. per cent. Phospholipid and free cholesterol possessed the same relative variation, the standard deviations being 17% of each of the respective means.

The expected range of two-thirds and of 95% of normal subjects have both been included in Table III. The range of 95% of cases should be used when interpreting the significance of a single analysis, the range of two-thirds of cases when comparing a group of values with the normal. It is rare to find the expected range of two thirds of cases of any condition exhibiting a lipaemia or a lipopenia to be much beyond the expected range of two thirds of normal subjects. Any group of values not thus overlapping in its expected two thirds range the corresponding range of normal subjects may be considered to have demonstrated a significant difference from normal. Actually the correct statistical conclusion to be drawn in such a case is that the expected range of two thirds of all cases of the values in the condition under study may be considered to lie beyond the expected range of two thirds of normal values.

## Lipids of the Red Blood Cells

The Interrelation of All Cases

A statistical analysis of the data obtained form 307 complete, differential lipid analyses of human red blood cells of normal and of pathological cases grouped according to the concentration of total lipid of each complete analysis has been presented in Table IV. These results have been condensed from

TABLE IV

The lipid composition of 307 samples of human red blood cells grouped according to the value for total lipid of each complete, differential analysis (The lipid values are expressed in mg. per 100 cc. of red blood cells)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos- pholipid	Free cholesterol	Cholesterol ester
		Minimum	300	0	156	69	0
		Maximum	399	104	292	128	43
300-399	7	Mean	354	40	209	94	11
		Standard deviation Standard deviation in per	51	39	46	22	15
		cent of mean	14%	98%	22%	23%	136%

TABLE IV-Concluded

The lipid composition of 307 samples of human red blood cells grouped according to the value for total lipid of each complete, differential analysis—Concluded (The lipid values are expressed in mg. per 100 cc. of red blood cells)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos- pholipid	Free cholesterol	Cholester
		Minimum	404	0	191	58	0
100 110	4.2	Maximum	446	169	344	118	74
400-449	13	Mean Standard deviation	431	26 46	296 40	93	16 27
		Standard deviation in per	15	40	40	19	21
		cent of mean	3%	177%	14%	20%	169%
		Minimum	450	0	198	62	0
		Maximum	499	110	391	179	99
450-499	65	Mean	477	38	301	106	32
		Standard deviation Standard deviation in per	16	35	38	18	24
		cent of mean	3%	92%	13%	17%	75%
		Minimum	501	0	109	73	0
		Maximum	546	262	417	150	147
500-549	63	Mean Standard deviation	527	62	313	111	41 34
		Standard deviation in per	22	48	51	19	34
		cent of mean	4%	77%	16%	17%	83%
		Minimum	550	0	231	68	0
		Maximum	595	233	453	220	182
550-599	49	Mean	573	52	353	128	40
		Standard deviation	14	54	50	28	40
		Standard deviation in per cent of mean	2%	104%	14%	22%	100%
		Minimum	600	0	284	79	0
		Maximum	646	191	478	173	230
600-649	25	Mean	627	64	384	126	53
		Standard deviation	14	59	34	22	27
		Standard deviation in per cent of mean	2%	92%	9%	17%	51%
		Minimum	651	0	237	85	0
650-699	23	Maximum Mean	692	63	540 430	189 136	172 41
030-099	23	Standard deviation	670	78	68	23	53
		Standard deviation in per	14	10	00	23	33
		cent of mean	2%	124%	16%	17%	129%
		Minimum	705	0	230	55	0
		Maximum	792	304	574	192	137
700-799	21	Mean	743	153	422	138	30
		Standard deviation	28	101	84	33	48
		Standard deviation in per cent of mean	4%	66%	20%	24%	160%
		Minimum	801	0	318	87	0
		Maximum	999	497	698	206	221
800-999	21	Mean	882	216	467	141	58
		Standard deviation Standard deviation in per	39	142	89	27	61
		cent of mean	4%	66%	19%	19%	105%
		Minimum	1000	0	272	50	0
		Maximum	1894	1380	864	217	391
Over 1000	18	Mean	1266	557	502	117	90
		Standard deviation	298	426	170	43	116
		Standard deviation in per	210		210	10	***

more than 3,000 lipid values. The data were divided into 10 groups of increasing total lipid values as indicated in the first column of Table IV. Although presented in a manner similar to those of plasma, it should not thus be inferred that the lipid values of the red blood cells bore a direct relation to the lipid content of plasma. It should not be inferred, in other words, that as the lipid content of plasma increased, so necessarily did the lipid content of the red blood cells. As a matter of fact such a correlation was seldom found.

The total lipid content of the red blood cells varied between 300 and 1894 mg. per cent. As in plasma, the percentage standard deviation was greatest in the groups with lowest total lipid values, except in the last group which included a wide range of very high values. The standard deviation varied from 2% to 23% of the respective means for total lipid. The total lipid content of the red blood cells is approximately the same in amount as that of plasma, covering likewise a range of values similar to that of plasma. There is, however, a marked difference in the composition of the total lipid of the erythrocytes from that of plasma. The lipids being in colloidal solution or bound in the structural make-up of the cell, are not readily diffusible across the red cell membrane and changes frequently occur in the percentage of plasma lipids without being reflected by corresponding changes in those of the red blood cells. The correct degree of such changes is not evident when whole blood is selected for lipid analysis, as is unfortunately often done. There are comparatively few situations in which an analysis of whole blood for lipids can be justified as preferable to an analysis of plasma or of serum. In any thorough study both plasma and the red blood cells should be examined.

Phospholipid may be seen from Table IV to be the lipid of greatest bulk in the red blood cells, and it is believed that this phospholipid is composed chiefly of cephalins and sphingomyelins. The concentration of phospholipid in the several groups was relatively constant, the standard deviation varying from 9 to 34% of the means. It would seem that most of the phospholipid of the red blood cells is structural rather than metabolic in function. Boyd and Tweddell (21) found no diurnal variation in the phospholipid content of the red cells, and Sinclair (32) was unable to detect elaidic acid in the phospholipid fatty acids of the red blood cells of cats after feeding this special type of fat.

Free cholesterol is, next to phospholipid, the lipid of greatest bulk in the red blood cells. The values of free cholesterol were somewhat more variable than those of phospholipid but less variable than those of neutral fat and cholesterol esters. The standard deviation ranged from 17 to 37% of the means. Free cholesterol, like phospholipid, is believed to be largely structural rather than metabolic in function in the red blood cells.

Values for *neutral fat* (Table IV) may be seen to have been found extremely variable. In every group, no matter what the value of total lipid, at least one analysis was recorded in which the neutral fat content was zero. The maximum values in each of the groups ranged from 100 to 300 mg. per cent,

except in the last two groups in which several cases with very high neutral fat values were encountered. The standard deviation extended from 66 to 177% of the means. It is obvious, therefore, that one should interpret with caution any relation between mean values for total lipid and mean values for neutral fat of the red blood cells. There is some evidence that the neutral fat of the red blood cells takes part in the lipid metabolism of the body (21).

The variations in the *cholesterol ester* content of the red blood cells were analogous to those of neutral fat (Table IV). In every group some analyses were found in which the cholesterol ester content was zero, although other members of the group had cholesterol ester values as high as from 43 to 391 mg. per cent. The standard deviation varied from 51 to 169% of the respective means. Thus one should again be cautious with cholesterol ester as with neutral fat in deciding upon any relation between mean values of this lipid and mean values of total lipid. Boyd and Tweddell (21) record evidence suggesting a diurnal variation in the cholesterol ester content of the red blood cells which would suggest that this lipid may also be concerned in sterol and lipid metabolism.

## The Relation of Component Lipids to Total Lipid of the Red Blood Cells

In Fig. 3 the mean values for the component lipids in the several groups listed in Table IV have been plotted against the mean values of the total lipid. As in Fig. 1, the expected range of 95% of normal total lipid values has been indicated. The values of the component lipids as they pass through

NORMAL RANGE

300 - PHOSPOLIPID

FREE CHOL® SON TOTAL LIPID

100 FOR ESTER AND TOTAL LIPID

TOTAL LIPID

100 FOR ESTER AND TOTAL LIPID

100 FOR ESTER AND TOTAL LIPID

100 FOR ESTER AND FOR ESTER AND

Fig. 3. The relation of mean values of lipids in the red blood cells of healthy and diseased persons to mean values of total lipid expressed in mg, per 100 cc.

the normal range of total lipid shown in Fig. 3 are not necessarily normal values for these lipids. The normal values are shown in relation to total lipid in Fig. 4.

Fig. 3 clearly demonstrates that the two chief lipids of the red blood cells under normal conditions, namely, phospholipid and free cholesterol, bear a similar relation to total lipid. The rate of increase for phospholipid was greater than that of free cholesterol as the total lipid increased. Values for both lipids bore an approximately direct linear relation to total lipid values until the total lipid exceeded about 900 mg. per cent, after which the increase was less for phospholipid and the free cholesterol content actually showed a decrease.

The marked increase in neutral fat of the red blood cells beyond the upper limit of the normal range of total lipid is due, as is seen from the results in Table IV, to the occurrence of many exceptionally high values in the analyses within the high groups. At the same time other samples of erythrocytes were found to contain small amounts of neutral fat or none at all in these same groups in which very high values occurred. The obvious conclusion from Fig. 3 that the neutral fat content of the red cells is markedly increased when the total lipid content rises very high, cannot therefore be made without reservation. Rather should it be said that with very high total lipid values in the red blood cells one may meet with exceptionally high neutral fat values but that such need not necessarily be found.

A similar situation prevailed with cholesterol ester values. Within the limits of the normal range of total lipid the mean values of cholesterol ester were low but tended to increase with increase in total lipid. There was an interesting analogy between the curve for cholesterol ester in Fig. 3 and the corresponding curve of Fig. 1, in that at and just above the upper limit of the normal range of total lipid, the curve for cholesterol ester of the red cells declined temporarily as it did in plasma. In view of the wide variation in values averaged in the mean and as given in Table IV, it is not possible to attach more than coincidental significance to these similarities. The increase in the mean values of cholesterol esters above the normal range cannot be taken as unqualified evidence that cholesterol ester always increases with increase in total lipid because of the likewise marked variation that was found at this time. It may be stated that high values for cholesterol ester are often found, but not necessarily so, when the lipid content of the red blood cells rises above the normal range.

## The Interrelation of Lipids of the Red Blood Cells in Normal Cases

Sixty-six samples of red blood cells from normal human adults were examined. The results obtained have been divided into six groups according to the total lipid values, and are presented in Table V. Variations in the

TABLE V
THE LIPID COMPOSITION OF 66 SAMPLES OF RED BLOOD CELLS FROM NORMAL HUMAN ADULTS GROUPED ACCORDING TO THE VALUE OF TOTAL LIPID OF EACH COMPLETE, DIFFERENTIAL ANALYSIS

(The lipid values are expressed in mg. per 100 cc. of red blood cells)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos- pholipid	Free cholesterol	Cholestero ester
		Minimum	425	0	239	90	12
		Maximum	442	71	302	103	66
400-449	4	Mean	435	21	280	95	49
		Standard deviation Standard deviation in per	9	29	23	5	33
		cent of mean	2%	138%	8%	5%	67%
		Minimum	454 .	0	243	79	0
		Maximum	493	110	293	116	99
450-499	13	Mean	477	68	264	97	48
		Standard deviation Standard deviation in per	14	33	32	14	26
		cent of mean	3%	49%	12%	14%	54%

TABLE V-Concluded

The lipid composition of 66 samples of red blood cells from normal human adults grouped according to the value of total lipid of each complete, differential analysis—Concluded

(The lipid values are expressed in mg. per 100 cc. of red blood cells)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos- pholipid	Free cholesterol	Cholestero ester
		Minimum	501	0	220	73	0
		Maximum	549	144	334	138	112
500-549	29	Mean	528	64	297	105	62
		Standard deviation Standard deviation in per	17	40	43	17	30
		cent of mean	3%	63%	14%	16%	48%
		Minimum	552	11	231	68	0
		Maximum	597	167	437	134	110
550-599	7	Mean	567	65	357	114	31
		Standard deviation Standard deviation in per	15	49	64	20	38
		cent of mean	3%	75%	18%	18%	123%
		Minimum	634	54	237	129	0
		Maximum	692	279	418	189	55
600-699	7	Mean	665	151	330	151	33
		Standard deviation Standard deviation in per	18	63	53	19	24
		cent of mean	3%	42%	16%	13%	73%
		Minimum	709	13	274	144	0
		Maximum	789	302	452	192	135
700-800	6	Mean	736	166	395	163	12
		Standard deviation Standard deviation in per	32	90	61	18	54
		cent of mean	4%	54%	15%	11%	450%

total lipid values were low in all groups. The standard deviation varied from 2 to 4% of the respective means for total lipid. The three lower groups contained zero values for neutral fat and in all groups the minimal value of this lipid was low. The standard deviation in the neutral fat values ranged from 42 to 138% of the respective means, a variation again sufficiently great to render dubious deductions regarding the relation of neutral fat to total lipid. The variations in the phospholipid values were much lower, the standard deviation extending from 8 to 18% of the means. The standard deviation represented 5 to 18% of the means for free cholesterol and hence mean values for both free cholesterol and for phospholipid may be taken as indicative of the trend of the values generally. An extremely wide range of values was recorded for cholesterol esters, the standard deviation being as phenomenally great as 450% of the mean in one group, showing that the means could hardly be regarded as indices for even a majority of values in a group.

With these variations in mind, Fig. 4 may be considered. In this figure the average values for the component lipids in each group have been compared with the means of the total lipid. Since the means for phospholipid and free

cholesterol may be taken as accurate indices of the changes taking place in the majority of samples, it is apparent that phospholipid and free cholesterol parallel each other in changes in concentration which bear roughly a direct linear relation to changes in total lipid. One cannot add from Fig. 4 any further deduction regarding the relation of neutral fat and cholesterol ester to total lipid other than has been expressed above, because of the marked variation in the values averaged in the means. On comparing Fig. 3 with Fig. 4 it is evident that the values of the component red cell lipids of both normal and of abnormal cases follow the same general course

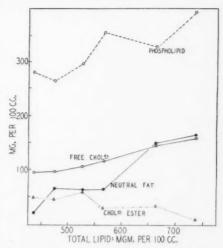


Fig. 4. The relation of mean values of lipids in the red blood cells of normal healthy human adults to mean values of total lipid expressed in mg. per 100 cc.

when passing through the range of normal total lipid values.

# The Normal Range of Lipids in the Red Blood Cells

To establish the normal range of values for lipids in the red blood cells of healthy normal human adults, as estimated by oxidative micromethods, the results in Table VI are offered. The minimal total lipid value encountered in the 66 cases studied was 425 mg. per cent and the highest 789 mg. per cent

TABLE VI
THE LIPID COMPOSITION OF THE RED BLOOD CELLS IN 66 COMPLETE DIFFERENTIAL
ANALYSES ON NORMAL HUMAN ADULTS
(The results are expressed in mg, per 100 cc, of red blood cells)

Value	Total lipid	Neutral fat	Phos- pholipid	Free cholesterol	Cholesterol ester
Minimum values*	425	0	220	68	0
Maximum values*	789	302	452	192	135
Mean values	523	84	311	115	13
Standard deviation	86	63	57	27	13 15
Standard deviation in per cent of mean, %	16	75	18	23	112
Expected range of 2/3 of normal subjects	437-609	21-147	254-368	88-142	0-28
Expected range of 95% of normal subjects	351-695	0-210	197-425	61-169	0-43

<sup>\*</sup>The sum of the individual lipids does not equal the total lipid because the individual lipid values are from different analyses.

with a mean of 523 mg. per cent and a standard deviation of 86, which was 16% of the mean. The range of 95% of total lipid values for the red blood cells was practically identical to that found in plasma, *i.e.*, roughly from 0.4 to 0.7%.

Total lipid of the normal human red blood cells was composed, on the average, of 59.5% of phospholipid, 22% of free cholesterol, 16% of neutral fat and 2.5% of cholesterol esters. The lipid composition of the red blood cells is thus considerably different from that of plasma. The red cells contain more phospholipid and free cholesterol and less neutral fat and cholesterol esters than does plasma. The average value for red cell neutral fat was 84 mg. per cent, the standard deviation being 63 which was 75% of the mean, and the expected range of 95% of cases was from 0 to 210 mg. per cent. The expected range of 95% of cases for phospholipid was 197 to 425 mg. per cent, for free cholesterol 61 to 169 mg. per cent and for cholesterol ester 0 to 43 mg. per cent.

## The Lipids of the White Blood Cells

The Interrelation of All Cases

In Table VII have been assembled the results of 174 complete, differential lipid analyses of human white blood cells, representing about 2,000 lipid values, grouped according to the concentration of total lipid. The lipid content of the white blood cells has been previously reported in a number of conditions (4, 6-8, 13, 16, 17, 20, 22) but has not previously been considered in relation to total lipid content. The results given in Table VII are expressed in mg. per 100 gm. of leucocytes, moist weight, and not in terms of mg. per 100 cc. of blood so that the lipid content of the leucocytes does not necessarily vary with the total leucocyte count.

There was little variation in the concentration of leucocytic total lipid, the standard deviations extending from 5 to 13% of the respective means of the several groups. Of the component lipids, phospholipid was most constant in value, the standard deviation being 19 to 43% of the means. The next least variation was in free cholesterol values, the percentage standard deviation varying from 21 to 38%. Neutral fat was quite variable with standard deviations extending from 63 to 125% of the means while cholesterol esters were most variable, the standard deviation ranging from 98 to 240% of the respective means.

Neutral fat and cholesterol ester have been shown to be the most variable of all lipids in each of plasma, the red blood cells and the white blood cells. A good deal of this variation is probably due to the fact that neither of these lipids are estimated directly but rather from two or more experimental figures. Cholesterol ester is calculated as the sum of ester cholesterol plus cholesterol ester fatty acid, which is calculated as 67% of ester cholesterol and this, in turn, is derived by subtracting the value of free cholesterol from that of total cholesterol, both of which are directly estimated. Neutral fats are calculated as 100/95 times the neutral fat fatty acids which are found by subtracting the values of the phospholipid and cholesterol ester fatty acids from that of

TABLE VII

The lipid composition of 174 samples of white blood cells of human blood grouped according to the value of the total lipid of each complete, differential lipid analysis

(The lipid values are expressed in mg. per 100 gm. of white blood cells, moist weight)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos- pholipid	Free cholesterol	Cholestero ester
		Minimum	501	0	257	82	0
		Maximum	798	244	552	220	139
500-799	21	Mean	667	68	433	146	20
		Standard deviation	89	73	92	31	48
		Standard deviation in per cent of mean	13%	107%	21%	21%	240%
		Minimum	800	0	313	22	0
		Maximum	1081	488	780	259	468
800-1099	32	Mean	985	180	550	160	95
		Standard deviation Standard deviation in per	95	144	117	53	103
		cent of mean	10%	80%	21%	33%	108%
		Minimum	1105	0	440	72	0
		Maximum	1392	642	1095	375	591
1100-1399	26	Mean	1250	216	681	208	145
		Standard deviation Standard deviation in per	117	184	185	63	164
		cent of mean	9%	85	27	30%	113%
		Minimum	1403	0	627	117	0
		Maximum	1679	638	1375	393	316
1400-1699	27	Mean	1537	165	1030	257	85
		Standard deviation	105	207	195	64	107
		Standard deviation in per cent of mean	7%	125%	19%	25%	126%
		Minimum	1726	0	269	63	0
		Maximum	1966	1004	1550	452	917
1700-1999	21	Mean	1900	361	1030	271	238
		Standard deviation Standard deviation in per	95	308	300	98	232
		cent of mean	5%	85%	29%	36%	98%
		Minimum	2015	0	411	129	0
		Maximum	2353	1390	1770	472	943
2000-2399	22	Mean	2174	527	1218	302	127
		Standard deviation in per	118	330	323	84	226
		cent of mean	5%	63%	27%	28%	178%
		Minimum	2423	0	823	245	0
		Maximum	2782	1460	2410	553	912
2400-2799	11	Mean	2591	442	1527	399	223
	1	Standard deviation in per	128	476	432	109	291
		cent of mean	5%	108%	28%	27%	131%
		Minimum	2586	0	354	171	0
		Maximum	3951	2650	2770	680	818
ver 2800	14	Mean	3360	1156	1586	385	233
		Standard deviation	287	943	680	147	252
		Standard deviation in per cent of mean	9%	82%	43%	38%	108%
	1	cent of mean	970	8270	4370	3870	108%

the total fatty acid. A summation of experimental errors must thus appear in the values of both neutral fat and cholesterol ester which may in a large part account for their variability.

In Fig. 5 the mean values of the component lipids have been plotted against the total lipid. Phospholipid and free cholesterol may be seen to bear a direct linear relation to total lipid as in the red blood cells, although the

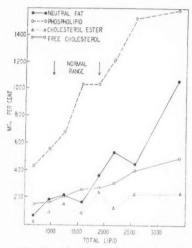


FIG. 5. The relation of mean values of lipids in the white blood cells of healthy and diseased persons to mean values of total lipid expressed in mg. per 100 gm. moist weight.

actual values of both lipids were much higher in the white than in the red blood cells. Considerable significance has been attached to the concentration of phospholipid in the white blood cells. The phospholipid content of a tissue appears to vary with its physiological activity, or tone, or functional capacity, or degree of training in the gymnastic sense. In conformity with this general hypothesis, Boyd (8, 13) has shown that increased values for phospholipid are found in the white blood cells of patients recovering normally from infection, while in patients who succumb to infection or combat it weakly, the phospholipid content of the white blood cells falls or remains low.

The increased amounts of neutral fat and cholesterol ester indicated by the rising mean curves in Fig. 5 cannot be taken as evidence that the values of

these lipids bear a direct relation to the concentration of the total lipid. In Table VII it was shown that the standard deviation of the means for these lipids was relatively high. Hence it may be concluded only that frequently high values for cholesterol ester and for neutral fat appear when the total lipid content rises above the normal range.

#### The Interrelation of Lipids of the White Blood Cells in Normal Cases

The variations encountered amongst the lipid values of the leucocytes from all types of cases were greater than those seen amongst the normal cases as listed in Table VIII. The standard deviation of the total lipid values for normal cases extended from 4 to 14% of the means, of neutral fat values from 46 to 126% of the mean, of phospholipid values from 11 to 22% of the means, of free cholesterol values from 19 to 44% of the means and of cholesterol ester values from 54 to 144% of the means respectively. It is evident from Fig. 6 that the behavior of normal values for component lipids within the normal range of total lipid was analogous to that of all cases presented in Fig. 5.

#### TABLE VIII

The lipid composition of 30 samples of white blood cells of normal human adults grouped according to the value of total lipid of each complete, differential analysis

(The lipid values are expressed in mg. per 100 gm. of white blood cells, moist weight)

Group (Total lipid)	No. of cases	Value	Total lipid	Neutral fat	Phos- pholipid	Free cholesterol	Cholestero ester
		Minimum	688	38	442	76	0
		Maximum	1076	397	630	242	306
To 1099	8	Mean	935	118	578	162	77
		Standard deviation Standard deviation in per	130	105	62	50	111
		cent of mean	14%	89%	11%	31%	144%
		Minimum	1128	0	457	72	14
		Maximum	1385	346	920	489	237
1100-1399	8	Mean	1250	173	704	265	108
		Standard deviation Standard deviation in per	117	138	152	63	98
		cent of mean	9%	80%	22%	23%	91%
		Minimum	1522	0	860	277	0
		Maximum	1894	595	1290	452	280
1400-1899	9	Mean	1740	164	1111	326	139
		Standard deviation Standard deviation in per	142	206	125	142	108
		cent of mean	8%	126%	11%	44%	78%
		Minimum	1963	61	990	245	0
		Maximum	2142	668	1380	420	155
Over 1900	5	Mean	2040	486	1112	327	115
		Standard deviation Standard deviation in per	74	223	146	61	62
		cent of mean	4%	46%	13%	19%	54%

The Normal Range of Lipids in the White Blood Cells

A composite statistical presentation of results for leucocytic lipids of normal human adults has been given in Table IX. The total lipid content of normal human white blood cells was calculated to have an expected range including 95% of cases from 603 to 2291 mg. per cent or approximately three times as great as occurs in plasma and the red blood cells. On the average the total lipid of the white blood cells was composed of 60% of phospholipid, 16.5% of free cholesterol, 14.5% of neutral fat and 9% of cholesterol esters. It is apparent from Table IX that single values for leucocytic neutral fat may be

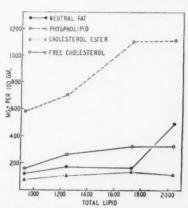


FIG. 6. The relation of mean values of lipids in the white blood cells of normal healthy human adults to mean values of total lipid expressed in mg. per 100 gm. moist weight.

TABLE IX

The lipid composition of the white blood cells in 30 complete differential analyses on normal human adults

(The results are expressed in mg. per 100 gm. of white blood cells, moist weight)

Value	Total lipid	Neutral fat	Phos- pholipid	Free cholesterol	Cholesterol ester
Minimum values*	688	0	442	72	0
Maximum values*	2142	668	1380	452	306
Mean values	1447	209	867	238	131
Standard deviation	422	212	281	95	121
Standard deviation in per cent of mean, %	29	101	32	40	92
Expected range of 2/3 of normal subjects	1025-1869	0-421	586-1148	143-333	10-252
Expected range of 95% of normal subjects	603-2291	0-633	305-1429	48-428	0-373

<sup>\*</sup>The sum of the individual lipids does not equal the total lipid because the individual lipid values are from different analyses.

considered normal if they occur within the range of from 0 to 633 mg, per cent. The corresponding expected range of 95% of values for phospholipid was from 307 to 1431 mg. per cent, for free cholesterol from 48 to 428 mg. per cent and for cholesterol ester from 0 to 363 mg, per cent.

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## STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

#### V. FURTHER PARASITES FROM THE OCELOT<sup>1</sup>

By THOMAS W. M. CAMERON<sup>2</sup>

#### Abstract

Several nematodes are recorded from the Ocelot (Felis pardalis); and the whipworm, Trichocephalus serratus, is described in detail.

In the third paper of this series, the parasites of various carnivores including an ocelot (Felis pardalis), were described. Recently the entrails of a second ocelot from Trinidad were sent by Professor F. W. Urich to the Institute of Parasitology. The animal proved to contain one species of Acanthocephala and three species of nematodes, all in small numbers.

## Echinopardalis pardalis (Westrumb, 1821) Travassos, 1918

One male and two females were recovered from the small intestine. The male measured 7.5 mm, and the females 8.5 mm, and 9.5 mm, respectively. The testes are elongated and situated one behind the other in the middle third of the body. The prostate glands are posterior to the second testis and are situated in pairs, although not so regularly as is described by Travassos (4). A very short neck is also present. These slight differences from Travassos' description however do not seem to be of specific importance, especially as the specimens are obviously young ones.

This acanthocephalid apparently is essentially a South American form and has been recorded from a number of Felidae (including the present host) on that continent. It has also been found in the domestic cat in North America.

The ocelot also contained a few examples of Ancylostoma caninum (Ercolani, 1859) and of *Molineus pardalis* Cameron, 1936, as well as several whipworms.

#### Trichocephalus serratus von Linstow, 1879

Two male, one immature and three mature female whipworms were found.

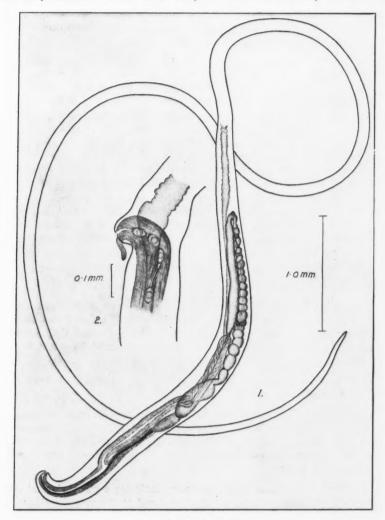
Both sexes are remarkable for their very small size and for the great length of the "neck". The mature females varied from 15.75 to 13.5 mm. in total length (av. 14.75) while the males were 12.5 and 13 mm, respectively. The average body length of the female was about 2.75 mm. and of the male, 3.4 mm. This gives a ratio of 5:1 for the length of "neck" to the body in the female and over 3:1 in the male. The females are 0.35 mm, wide and the males 0.3 mm. The head end in each sex is similar and typical. There

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is a single broad, bacillary band present in the "neck", originating close to the head and terminating about the level of the body. The remaining part of the "neck" and all the body is finely striated transversely.

The female is a more or less typical whipworm, the vulva being situated at the junction of "neck" and "body". From the lower lip of the vulva



Trichocephalus serratus.

FIG. 1. Entire male whipworm.

Fig. 2. Vulvar region of female.

however (Fig. 2) there projects a smooth finger-like process. The female genital system is typical. The ovarian tubule originates close to the anus and passing forward to the level of the vulva, turns back again to just anterior to its origin. There it turns and becomes a voluminous uterus which opens at the vulva. In the young specimen, this uterus appeared to be divided into two parts, but no such division was noticeable in the adults. In this same specimen, there were two pairs of minute sessile papillae just anterior to the anus. The eggs are of the typical whipworm type, show little variation in size, and measure (in utero)  $60\mu \times 35\mu$ .

The male (Fig. 1) has no terminal spiral and superficially resembles the female. Unfortunately in both cases the spicule was retracted but both specimens were well preserved and the details clearly visible. However, it is not possible to say whether or not the "sheath" is campanulate when extruded. It is spinose, and when retracted is about 0.4 mm, in length. The spicule, 1.2 mm. long, is simple and filiform. Its anterior end is attached to a large retractor muscle. About half way down the spicular canal, it is joined by a voluminous muscular tube which splits at its anterior end to join the rectum and male duct. This cloacal tube is presumably contracted towards the rear end of the body when the "sheath" is extruded. The male genital opening is dorsal to the rectum and a short canal joins the cloaca with a massive cement gland. This in turn communicates with the seminal vesicle by a narrow opening. The seminal vesicle runs as far as the junction of intestine and oesophagus where it communicates with the single testicle. This is a wide, laterally convoluted tube, which originates just posterior to the junction of the male and digestive tubes.

The digestive system is quite typical.

Whipworms are somewhat rare in Felidae and such records as exist all refer to South American cats. The sole reference to whipworms from this particular host is one by Canavan (2) who found a single female 42 mm. long in an ocelot which had died after a short period of captivity in the United States.

In 1851, Diesing recorded *T. felis* from *Felis tigrina* in Brazil but his species is not recognizable.

In 1879, von Linstow described *Trichocephalus serratus* from the domestic cat, and in 1889 he described *T. campanulus* from the same host; both examples came from Brazil. In 1923, Urioste examined some specimens from the cat from the same country and concluded a single species existed: this he called *Trichocephalus serratus*.

There are few details in this genus available for a morphological comparison of species and resource has to be made to the very unsatisfactory criterion of measurements.

	T. serratus	T. campanulus	T. serratus	Present
	(von Linstow)	(von Linstow)	(Urioste)	species
Male	40.0 mm.	Not stated	20.5 mm.	12.75 mm.
Spicule	3.9 mm.	Not stated	1.5 mm.	1.2 mm.
Female	48.0 mm.	31.5 mm.	23.0 mm.	14.75 mm.
Eggs	56 × 39μ	72 × 36µ	80 × 36 $\mu$	60 × 35µ

T. serratus is stated to have a vaginal prolapse while in T. campanulus the vulvar lips are slightly salient; the projecting lower vulvar lip in the present species is no doubt the same structure interpreted by von Linstow as a prolapse. The differences between the present species and T. serratus are accordingly mainly differences in dimensions, those for body and spicular lengths of the present specimens being roughly one-third of T. serratus; the eggs however are almost identical in size. There seems no reason accordingly, why it should not be referred to von Linstow's first species. While the present evidence is too scanty to allow of a definite opinion, it seems probable that only a single species exists in Felidae. It is peculiar however that all records of whipworms in cats should be South American.

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